University of Alberta

SUPPLEMENTATION OF MICROBIAL PHYTASE TO DIFFERENT SWINE DIETS: EFFECT ON THE UTILIZATION OF VARIOUS NUTRIENTS

By

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ABSTRACT

The very limited ability of pigs to utilize phytate phosphorus (**P**) in feedstuffs of plant origin poses environmental and nutritional problems. The objective of this research was to determine the effect of phytase supplementation to swine diets on the utilization of various nutrients. In phase 1 study, four experiments were conducted with weanling pigs fed four practical diets, respectively. In phase 2, one experiment was conducted with growing pigs fed two model (i.e. a high- and a low-phytate) diets.

The supplementation of phytase to six different diets improved ($P < 0.001$) the apparent fecal digestibility (**AFD**) of P by 6.5 to 16.9 percentage units (**pu**). The fecal output of P was reduced ($P < 0.05$) by 9.0 to 30.5%, and the P retention was improved ($P < 0.05$) by 12.7 to 50.2%. The AFD of Ca increased ($P < 0.05$) with the wheat-soybean meal and the barley-peas-canola meal diets, but not with the other two practical diets fed to weanling pigs. In growing pigs, the AFD of Ca increased ($P < 0.05$) with the low-phytate diet, but not ($P > 0.10$) with the high-phytate diet. The Ca retention was increased ($P < 0.05$) with one of the four practical diets. There was no effect ($P > 0.10$) of phytase supplementation on the AFD of Cu, Fe, Mg, Mn, and Mo.

No significant improvements ($P > 0.05$) in the apparent ileal digestibilities (**AID**) of CP and amino acids (**AA**) were detected upon phytase supplementation to six diets. However, small numerical increases were consistently found. The increases for some AA approached significance ($P < 0.10$). Numerically, the AID of CP was increased by 0.2 to 2.8 pu, and the average of the AID of the indispensable AA was increased by 0.5 to 2.9 pu.

The increases ($P < 0.05$) in the AFD of ash were detected in four of the six diets. The AFD of DM tended to increase ($P < 0.10$) in the wheat-soybean meal and the barley-peas-canola meal diets fed to weanling pigs. There was no improvement ($P > 0.10$) in the AFD of energy upon phytase supplementation.

DEDICATION

To my family!

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LIST OF ABBREVIATIONS

Abbreviations	Definitions
AA	Amino Acid(s)
ADFI	Average Daily Feed Intake
ADG	Average Daily Gain(s)
AFD	Apparent Fecal Digestibility(ies)
AID	Apparent Ileal Digestibility(ies)
ANOVA	ANalysis Of VAriance
BW	Body Weight
Ca	Calcium
CP	Crude Protein (N × 6.25)
Cu	Copper
d	day(s)
DE	Digestible Energy
DM	Dry Matter
DP	Digestible Phosphorus
Exp.	Experiment(s)
FCR	Feed Conversion Ratio
Fe	Iron
FTU	Phytase Unit(s)
g	gram(s)
GMO	Genetically Modified Organisms
h	hour(s)
i.d.	inside diameter
kcal	Kilocalorie
kg	kilogram
ME	Metabolizable Energy
min	minute(s)

Mg	Magnesium
mL	milliLitre
mM	milli-Molar
mmol	millimole
Mn	Manganese
Mo	Molybdenum
N	Nitrogen
N	Normal distribution
n	sample size
NDF	Neutral detergent fiber
NE	Net Energy
P	Phosphorus
P	Probability
ppm	parts per million
pu	percentage unit(s)
r	Simple correlation coefficient
RSD	Relative Standard Deviation
SAS®	Statistical Analysis System
SD	Standard Deviation
SEM	Standard Error of the Mean
vol	volume
vs.	versus
wk	week(s)
Zn	Zinc

CHAPTER I. LITERATURE REVIEW [1]

A. Introduction

It is well known that even in modern animal production there are still considerable proportions of nutrients in feedstuffs that cannot be digested and/or absorbed by the animal. Phytic acid/phytate is one of the most significant anti-nutritional factors present in feedstuffs of plant origin for nonruminants. The very limited ability of nonruminants to utilize phosphorus (**P**) in phytate poses three problems to environmentalists, animal producers, feed manufacturers, and the general public as well. The first problem concerns the environmental impact resulting from P (actually phosphate) excretion in manure. Growing concerns over this issue have been expressed, especially in Europe. The second problem involves the formulation of diets that satisfy the requirement of animals for P, taking into account that phytate-P is of low bioavailability. The third problem relates to the ability of phytic acid/phytate to form complexes with dietary nutrients including minerals, proteins, free amino acids (**AA**), and starch (Ravindran et al., 1995; Sebastian et al., 1998; Jongbloed et al., 2000a).

In order to meet the P requirement of nonruminants, nutritionists usually supplement diets with inorganic P sources such as monocalcium phosphate or dicalcium phosphate. This is not only expensive, but also fails to address the environmental pollution resulting from P. On the other hand, phytic acid/phytate can also supply P to the animal, if the feedstuffs or diet is treated properly. Recent legislation in some countries and some states in the USA has forced the feed industry to look for alternative ways to make phytate-P more available to animals. As results of nutritional significance and environmental concerns, there is renewed interest in using exogenous phytase to

[1] The first version of this review was published in "Food Science and Product Technology", a book edited by T. Nakano and L. Ozimek, 2002, pages 199-227. Research Signpost, Kerala, India.

solve the aforementioned P-related problems. In addition, dietary supplementation of phytase may also improve the bioavailabilities of some other nutrients, and diminish the excretion of phytic acid/phytate-bound nutrients such as nitrogen (N) and minerals other than P (Jongbloed and Lenis, 1998; Kornegay, 1999). In short, the total nutritional value of feedstuffs and the performance of pigs may be improved by phytase supplementation (Wenk et al., 1993; Sebastian et al., 1998, Ludke et al., 2000). Presently, phytase may well be considered a "miracle enzyme" just as soybeans were described as a "miracle crop" for producing high quality protein in the 1960's (Kornegay, 1999).

As to the implication of phytate and dietary supplementation of phytase in animal production, excellent reviews were published including those by Nelson (1967), Ravindran et al. (1995), Sebastian et al. (1998), and Zyla (2001) in the field of poultry nutrition, and by Kornegay (1999; 2001) in the field of nonruminant nutrition. In the field of swine nutrition/production, one review was published in 1993 and updated in 2000 by Jongbloed et al. (1993, 2000a). The objective of this review is to summarize current knowledge on the supplementation of microbial phytase to swine diets, with emphasis on the utilization of nutrients such as P, other minerals, crude protein (**CP**), AA, and starch. Where possible, tentative explanations for diverse, contradictory, or controversial results from the literature were provided. Furthermore, factors influencing the efficacy of microbial phytase were discussed.

B. Phytic Acid, Phytates, Nutrients and Phytases

Phytic Acid, Phytates and Nutrients

Phytic acid (myo-inositol hexaphosphoric acid, $C_6H_{18}O_{24}P_6$, MW = 659.9), scientifically referred to as 1, 2, 3, 4, 5, 6-hexakis dihydrogen phosphate myo-inositol, is an anti-nutritional constituent abundant in many plant materials used as animal feeds or human foods (Maga, 1982; Graf, 1986; Reddy et al., 1989). The content of this acid in plant products differs considerably, varying from 0.004% in wheat endosperm to 6.39% in corn germ (Reddy et al., 1989; Zyla, 2001). It usually comprises about 0.5 to 4.5% of most cereal grains, legumes, and oilseed meals that are commonly used as feedstuffs for

swine (Table I-1). Phytic acid, having six reactive phosphate groups, is strongly negatively charged in a soluble form over a wide range of pH, from 3.5 or less to 9.0 or higher (Reddy et al., 1989). The potential for binding positively charged molecules such as cations, starch, or proteins/AA below the isoelectric point is tremendous. The binding can possibly occur within one phosphate group or between two phosphate groups on either one or more phytic acid molecules, resulting in very complicated chelate compounds (Reddy et al., 1989; Jongbloed et al., 2000a). The insoluble salt form of phytic acid is more stable than the acid form, and is commonly referred to as phytate, or scientifically as the hexakis-phosphoric acid ester of myo-inositol (Maga, 1982; Graf, 1986). One model of a molecular structure of phytic acid/phytate and its possible interactions with some nutrients are presented in Figure I-1. Actually, a great variety of phytate-nutrient complexes can be found in plant material, because various minerals, proteins, AA, and starch can be bound. Quite often, phytates are present in forms of mono to dodeca anions of phytic acid, of which most are the lower phosphate derivatives or esters than 1, 2, 3, 4, 5, 6 - hexaphosphate. All these polyphosphorylated inositols are usually referred to as phytates (De Boland et al., 1975; Maga, 1982). In a recent study, however, Kemme (1998) found that most phosphates were in the form of hexaphosphate (70 to 80%). The fraction of tetraphosphate was in general less than 0.05% of the hexaphosphate. In the literature, the name phytic acid has been used interchangeably with the term phytate, while the calcium-magnesium-potassium salt of phytic acid is referred to as phytin. (Reddy et al., 1989). In this review, the term "phytate" means phytic acid and/or phytate residues.

Ubiquitous in nature, phytates serve several physiological functions for living organisms, such as nutrient storage and antioxidant protection for plants (Cosgrove, 1980; Reddy et al., 1989; Ravindran et al., 1995). The phytate contents in many feed ingredients have been determined (Nelson, et al., 1968; De Boland et al., 1975; Kirby et al., 1988; Reddy et al., 1989; Eeckhout and De Paepe, 1994; Ravindran et al., 1994, 1995; Tyagi et al., 1998). The average values for feedstuffs of plant origin that may be included in swine diets are summarized in Table I-1, which shows that the phytate-P contents range from 0.01% in alfalfa meal to 1.07% in rice bran. It was also cited in the literature

(Mroz et al., 1994; Kemme, 1998) that feed ingredients of plant origin may contain from 0.7 to 3.5% phytate.

Phytic acid and phytate residues in animal diets or human foods are thought to have a strong capacity to bind many nutrients including minerals, AA, proteins, and starch. Phytate-bound nutrients are very poorly available for absorption in nonruminants, and excreted in manure. In addition, phytic acid or phytate may also bind some digestive enzymes, such as α-amylase and proteases, in the digestive tract of pigs and render these enzymes less effective (Mroz et al., 1994; Szkudelski, 1997). On the subject of phytic acid and/or phytates, several reviews and books have been published, in which their chemistry, biochemistry, occurrence in plants, methods of analysis, and implications in human nutrition and medicine were discussed (Cosgrove, 1966; Cheryan, 1980; Cosgrove, 1980; Maga, 1982; Reddy et al., 1982, 1989; Wise, 1983; Graf, 1986; Szkudelski, 1995, 1997; Maenz, 2001).

Phytases

Phytases (myo-inositol hexaphosphate hydrolases) are enzymes that catalyze the dephosphorylation of phytate complexes by cleaving the chemical bonds within or between phytate molecules, resulting in stepwise removal of orthophosphate groups from phytates (Maga, 1982). This process allows the bound nutrients to be released from phytates for animal utilization. Present in microorganisms, plants and certain animal tissues are two main types of phytases, which have been recognized by the International Union of Pure and Applied Chemistry and the International Union of Biochemistry: 3-phytase (EC 3.1.3.8) which firstly hydrolyzes the phospho-ester bond at position 3 of myo-inositol, and 6-phytase (EC 3.1.3.26) which initiates the hydrolysis of phytate at position 6 (Graf, 1986). Eventually, both phytases will fully hydrolyze phytate to inorganic phosphate and myo-inositol via intermediate forms of myo-inositol ortho-phosphates (i.e. penta- to mono-phosphates) (Graf, 1986; Ravindran et al., 1995). The 3-phytase enzyme is characteristic for microorganisms and has been found most frequently in *Aspergilli*, a genus of ascomycetous fungi. The 6-phytase enzyme is typically found in higher plants. Rye, triticale, wheat, and barley contain relatively high levels of phytase activity, while those in corn, oats, rice, sorghum, oilseed meals, and legumes are very low

or absent (Table I-1). It was reported that phytase activity is not related to the total P or phytate-P content in the feed ingredients (Eeckhout and De Paepe, 1994; Barrier-Guillot, et al., 1996). Intrinsic phytase activities in nearly all feedstuffs of plant origin, however, are not sufficient for livestock to hydrolyze phytate efficiently. Microorganisms in the gastrointestinal tract of ruminants can synthesize sufficient endogenous phytases for animal utilization. The activity of endogenous phytase in the gastrointestinal tract of nonruminants, however, is negligible (Reddy et al., 1989; Cromwell et al., 1993; Kornegay, 2001). The supplementation of exogenous phytase to diets for nonruminants, therefore, must have beneficial effects on animal performance by releasing the bound nutrients from phytate. As to phytase activity, at least four units are used in the literature: FTU, PTU, PU, and U, which are not always the same, especially in publications prior to 1994. The activity of one FTU or PTU is usually 1000 times higher than of one PU or U. In this review, phytase activity is expressed as **FTU** (phytase units). Values expressed as PTU, PU, and U in the literature were converted to FTU according to their definitions in the respective papers. One FTU is defined as that amount of phytase that liberates 1 mmol of ortho-phosphate per minute from 5.1 mM Na-phytate at pH 5.5 and 37°C (Eeckhout and De Paepe, 1994; Engelen et al., 1994, 2001). The 3-phytase enzyme (EC 3.1.3.8) produced by *Aspergillus* (*A. ficuum*) is a glycoprotein with a molecular mass between 85 to 100 kDa. The optimum temperature for the activity of this enzyme is between 60 to 70°C. It has two pH optima: one at pH 2.5 and the other at pH 5.5. More detailed information regarding research and application history, chemical properties, and mode of action of microbial phytase can be found in reviews by Nayini and Markakis (1986), Newman (1991), Wodzinski and Ullah (1996), Wyss et al. (1999a), Maenz (2001) and Zyla (2001).

Industrial preparations of phytase are obtained either by extraction of plant or animal tissues, or by fermentation of suitable microorganisms. The latter preparation, referred to as microbial phytase, is presently the main industrial source. Eeckhout and De Paepe (1991) found that microbial phytase was 74% more efficient than phytase in wheat middlings in a study with pigs when added at equal *in vitro* activity levels (cited from Kornegay, 1999; 2001). The use of microbial phytase as a supplement to nonruminant diets is not a new concept. In the late 1960's and early 1970's, studies by Nelson et al.

(1967, 1968, 1971) showed that phytases produced from *Aspergillus ficuum* and other molds were effective in improving P bioavailability from phytate for chickens. However, supplementation of phytase to nonruminant diets at that time was not cost-effective. Recent advances in biotechnology including recombinant DNA and microbial fermentation technology have allowed enzyme manufacturers to produce phytase efficiently on a large scale (Cromwell, 1991; Kornegay, 1999). Although commercial phytases have been available for many years, not until the environmental impact of agriculture and urban sprawl became a political issue was there pressure to develop and supplement microbial phytase to feedstuffs for livestock (Roland et al., 2000). The mandate in The Netherlands given to livestock producers to use phytase to reduce P excretion was one of the driving forces (Kornegay, 1999). Presently available in the marketplace are several distinct preparations of phytase, including Natuphos®, Finase™, Phytase Novo®, Biofeed Phytase®, and Allzyme Phytase™, in either liquid, dry powder, granular or coated forms. However, patents still affect the availability of these products in different countries (Kornegay, 2001; Zyla, 2001). Natuphos® is the first and most widely used phytase in the feed industry, and is produced from *Aspergillus niger* var. *van tieghem* (a species of fungi, formerly known as *A. ficuum*) by DSM (formerly Gist-brocades), The Netherlands, and distributed by BASF, Germany. Finase™, another commercial preparation, is produced from *Trichoderma reesei* and manufactured by Röhm Enzyme Finland Oy (Zyla, 2001). Finase-F and Finase® FP500 are two products produced by Alko Ltd. Biotechnology, Finland. Phytase Novo® is a product of Novo Nordisk Company, Denmark. Almost all these preparations are obtained by fermentation of genetically modified/mutant strains of *Aspergillus niger*. Allzyme Phytase™ from Alltech Inc., the United States is claimed to be the only preparation derived from a non-genetically modified strain of *Aspergillus niger* (Cromwell et al., 1995a; Roland, et al., 2000; Zyla, 2001). Biofeed phytase®, a 6-phytase enzyme from Novo-Nordisk, originates from another species of fungi, *Peniophora lycii* (Zyla, 2001). Toxicity trials showed that microbial phytase from genetically-modified fungi fed to pigs and poultry at 5- to 20-fold higher than the recommended level (500 FTU/kg diet) had no adverse effects on animal health based on the results from a general necropsy and histological

examination of liver, kidney and tibial tissues (Kornegay, 1999; 2001; Zhang et al., 2000).

C. Effect of Phytase on Phosphorus Bioavailability

It is recognized that approximately two-thirds of total P in feedstuffs of plant origin is not available to nonruminants. This assumption is based on the fact that the majority of P is in the form of phytate-P and only about one-third in the form of non-phytate P (Cromwell, 1992; Weremko et al., 1997). Phytate-P, which is of very limited bioavailability to nonruminants, usually accounts for 60 to 90% of the total P in cereal grains, oilseeds (oilseed meals), and legumes (Graf, 1986; Jongbloed et al., 2000a), and for 4 to 81% in feedstuffs for swine (Table I-1). The poor digestibility of phytate-P in feedstuffs of plant origin results in a large quantity of P excreted in swine manure, which can create serious environmental pollution, especially in parts of the world where land and water resources are scarce and animal production is intensive (Cromwell, 1991; Cromwell and Coffey, 1991).

Effect of Phytase on P Bioavailability

Commercial preparations of microbial phytase were initially developed to increase the digestibility/bioavailability of phytate-P in feedstuffs of plant origin for nonruminants in order to reduce the animal reliance on inorganic P supplementation, decrease the P output in manure, and alleviate P pollution of the soil and water. Numerous investigations with pigs have shown that supplementation of microbial phytase to swine diets can achieve the aforementioned goals (Simons et al., 1990; Näsi, 1990; Cromwell, 1991; Beers and Jongbloed, 1992a, b; Jongbloed et al., 1992; Kemme and Jongbloed, 1993; Cromwell et al., 1993; 1995a, b; Lei at al., 1993b, c; Ketaren et al., 1993; Young et al., 1993; Mroz et al., 1994; Pallauf et al., 1994; Näsi and Helander, 1994; Näsi et al., 1995; Yi et al., 1996; Biehl and Baker, 1996; Chiang and Hwang, 1997; Han et al., 1997; Harper et al., 1997; Murry, et al., 1997; O'Quinn et al., 1997; Valaja et al., 1998; Kemme et al, 1999b; Kornegay, 1999; Traylor et al., 2001). A comprehensive review on the

effect of phytase on P digestibility in pigs was provided by Düngelhoef and Rodehutscord (1995).

Since the mid 1980's, a series of experiments were carried out at the Institute for Animal Science and Health (ID-DLO), Lelystad, The Netherlands on supplementation of microbial phytase from *Aspergillus niger* to diets for nearly all categories of pigs. Simons et al. (1990) initially reported that a crude phytase preparation supplemented to a corn-soybean meal diet (1000 FTU/kg diet) for growing pigs increased the apparent P digestibility by 24 percentage units (**pu**); the amount of P in feces was reduced by 35%, which implied that about 50% of P from phytate was made available for utilization. Jongbloed et al. (1992) carried out studies with cannulated growing pigs fed a corn-soybean meal diet and a typical Dutch diet. Upon phytase supplementation (1500 FTU/kg diet), 60 to 74% of phytic acid was hydrolyzed before reaching the distal end of the small intestine. The apparent ileal digestibility (**AID**) of P was increased by 18.5 to 29.8 pu; the apparent fecal digestibility (**AFD**) of P by 27.0 to 29.7 pu. Beers and Jongbloed (1992a) reported that the AFD of P was increased by 21.6 pu when phytase (1450 FTU/kg diet) was supplemented to a corn-barley-soybean meal diet for weanling pigs. Kemme and Jongbloed (1993) reported that the AFD of P was increased by 18 pu in growing pigs fed a corn-tapioca-peas-wheat bran-soybean meal-sunflower seed meal diet supplemented with microbial phytase (500 FTU/kg diet). Mroz et al. (1994) reported that the AID of P was increased by 25.8 pu and the AFD of P by 24.1 pu in a corn-tapioca-soybean meal diet supplemented with phytase (800 FTU/kg diet) for growing pigs. In another study, Kemme et al. (1999b) reported that supplementation of Natuphos® (900 FTU/kg diet) increased the AFD of P by 16.2 pu for growing-finishing pigs fed a corn-soybean meal diet. Similar results on P digestibility in studies with Natuphos® supplementation were also found in many other countries (Wodzinski and Ullah, 1996), including Australia (Ketaren et al., 1993), Finland (Näsi and Helander, 1994; Valaja et al., 1998), Germany (Pallauf et al., 1994), and the United States (Cromwell et al., 1995b; Yi et al., 1996; Liu et al., 1996, 1997, 1998; Murry, et al., 1997; O'Quinn et al., 1997; Harper et al., 1997; Traylor et al., 2001), to name a few.

Another phytase product, Finase-F, was also evaluated with growing pigs fed a corn-soybean meal diet by Näsi (1990) in Finland. Supplementation at 100 to 500

FTU/kg diet enhanced the AFD of P by 5 pu. Cromwell et al. (1993) in the United States reported that Finase™ (1000 FTU/kg diet) increased the P bioavailability from 25 to 57% in a soybean meal diet, and from 15 to 43% in a corn-soybean meal diet fed to growing-finishing pigs. At this level of phytase supplementation, approximately one-third of unavailable P was made available. Young et al. (1993) reported that Finase-F supplementation, at rates of 500 and 1000 FTU/kg diet, increased the AFD of P by 8 and 11 pu, respectively. Lei et al. (1993b) reported that Finase-F (750 FTU/kg diet) increased P retention by 50% and decreased fecal P excretion by 42% in weanling pigs fed a corn-soybean meal diet. Finase-F supplementation, over a range from 0 to 750 FTU/kg diet, resulted in a linear improvement in P utilization. Näsi et al. (1995) reported that Finase® FP500 supplementation to a barley-rapeseed meal diet resulted in an improvement in fecal P digestibility from 36 to 45% ($P < 0.001$). Supplementation of Allzyme Phytase™ (a preparation with moderate activity, 50 FTU/g) in improving phytate-P bioavailability for growing pigs fed a corn-soybean meal diet was also reported by Cromwell et al. (1995a). Li et al. (1998) found that the AFD of P was improved ($P < 0.05$) by supplementation of Phytase Novo® (750 FTU/kg diet). A nonlinear response of supplemental phytase (X: FTU/kg diet) on P digestibility (Y: %) for pigs (Figure I-2) was generated by Kornegay et al. (1998) based on results compiled from 52 experiments in the literature (representing 32 references) (Kornegay, 1999):

$$Y = 54.86\ (1 - 0.4908e^{-0.00263X}),\ r^2 = 0.47$$

Phytase and P Equivalence

The efficacy of microbial phytase has been compared to that of inorganic P. The P phytase equivalency value, a term used to describe the replacement value of P, is defined as the amount of inorganic P which does not have to be included into the diet when a given amount of phytase is supplemented (Kornegay, 1999). Beers and Jongbloed (1992b) quantified the amount of digestible phosphorus (**DP**) liberated by phytase in a dose-response study with growing pigs. Six doses of Natuphos® (0, 200, 400, 700, 1000 and 2000 FTU/kg diet) were supplemented to two types of diets. Diet A contained corn and soybean meal as main ingredients; Diet B, in addition, contained some phytate-rich by-products. Both diets contained low levels of P (3.1 to 3.8 g/kg). The phytase efficacy

appeared to be related to the dose supplied and the type of diet used, as shown in Figure I-3. The relationships were illustrated by an exponential and a logistic curve for these two diets, respectively, with the following equations (Jongbloed et al., 2000a):

DP (g/kg) = $1.86 - 1.0013 \times 0.9963^{dose}$ ($r^2 = 0.967$; RSD = 0.067 g/kg), and

DP (g/kg) = $0.95 + 1.31/(1 + e^{(-5.51 \times 10^{\wedge}-3(dose - 377.8))})$ ($r^2 = 0.955$; RSD = 0.092 g/kg)

Although there is a slight difference in the shape of the two curves (Figure I-3), in both diets DP was increased by approximately 0.8 g/kg diet at the inclusion rate of 500 FTU/kg diet, which appears to be the optimal dose response. In most cases the dose-response relationship can be best described by an exponential curve (Düngelhof and Rodehutscort, 1995; Kornegay, 1999), which can also be concluded from the studies by Cromwell et al. (1995a) with Allzyme Phytase™ (cited in Jongbloed et al., 2000a). Traylor et al. (2001) reported that the AID of P increased quadratically ($P < 0.01$) with increasing levels of Natuphos® supplementation (from 0 to 1500 FTU/kg diet). While a maximum response is achieved at up to 1000 FTU/kg diet, a dose of 400 to 500 FTU/kg diet appears to give the optimum response (Khan, 1995). Under some conditions, the addition of more than 500 FTU/kg diet may be advantageous, but there will be a lower P-equivalence value. Obviously, a significant interaction exists between the total P level and the level of phytase supplementation (Cromwell et al., 1993; Näsi et al., 1995).

Based on P digestibility studies, as well as P retention and bone ash, it was calculated that 1 g of inorganic P from monocalcium phosphate was equivalent to 500 FTU (Kies et al., 2001). Lei et al. (1993c), using weanling pigs, reported that one FTU supported retention of 1.1 mg of P from a corn-soybean meal diet, which was equivalent to 0.91 mg of inorganic P from mono-dibasic calcium phosphate. Kornegay and Qian (1996) derived an average P equivalency function (Y: P g/kg diet) with phytase supplementation (X: FTU/kg diet) at available P levels of 0.07% and 0.16%: $Y = 2.622 - 2.559e^{-0.00185X}$. Using this function, it can be predicted that it would require about 246 FTU to replace 1 g of inorganic P from defluorinated phosphate, which represents 41% of P released from phytate. In the same year, Yi et al. (1996) published another average P equivalency function with phytase supplementation at available P levels of 0.05% and 0.16%: $Y = 1.546 - 1.504e^{-0.0015X}$. From this function, it can be predicted that it would

require approximately 676 FTU to replace 1 g of inorganic P from defluorinated phosphate, which represents 77% of P released from phytate. Since the phytase equivalency value for total P or DP is a variable dependent on the inorganic P source that is replaced, it should always be kept in mind that the equivalency value used in practice must be adjusted by the apparent digestibility value (Kornegay, 1999).

The aforementioned results indicate that phytase, if used correctly, can largely or entirely replace inorganic P that is usually supplied to swine diets (Lei et al., 1993c; Khan, 1995; Han et al., 1997). A fundamental economic benefit that microbial phytase can deliver is its ability to replace the inorganic P source required for diet formulation (Kies et al., 2001). Although the P requirement of weanling pigs fed supplemental phytase (500 and 1000 FTU/kg diet) has been estimated by Roberson (1999), one issue that still needs to be addressed is the amount of phytase and/or inorganic P source that are needed for different categories of pigs under different dietary conditions.

D. Effect of Phytase on the Bioavailabilities of Other Minerals

Phytic acid and phytate residues in animal feeds or human foods may bind polyvalent cations, like calcium (Ca^{2+}), zinc (Zn^{2+}), copper (Cu^{2+}), magnesium (Mg^{2+}), manganese (Mn^{2+}), iron ($Fe^{2+/3+}$), molybdenum ($Mo^{2+/3+}$), cobalt (Co^{2+}) and selenium (Se^{2+}), forming insoluble phytate-mineral complexes, rendering these minerals poorly available for nonruminants or humans (Wise, 1983; Reddy et al., 1989; Adeola et al, 1995; Szkudelski, 1995; Jongbloed et al., 2000a). Using *in vitro* potentiometric titration methods, the solubility and relative stabilities of various phytate-mineral (metal) complexes were studied. Vohra et al. (1965) indicated a descending order of formation of phytate-mineral complexes at pH 7.4 as follows: $Cu^{2+} > Zn^{2+} > Ni^{2+} > Co^{2+} > Mn^{2+} > Fe^{3+} > Ca^{2+}$. Whereas Maddaih et al. (1964) found the descending order of salt stability to be: $Zn^{2+} > Cu^{2+} > Co^{2+} > Mn^{2+} > Ca^{2+}$ at physiological pH. Zinc and Cu appear to have the highest affinity for phytate to form complexes, while Ca has the lowest affinity. Whether or not a particular salt is formed depends on the pH, the phytic acid level, the mineral concentration, and the presence of other cations. The possible synergistic effect of two or more cations which may co-precipitate to increase the quantity of phytate-

mineral complex is already known (Maga, 1982; Ravindran et al., 1995); however, the *in vivo* mechanism by which phytate affects mineral nutrition is not completely understood yet (Reddy et al., 1989). Most *in vivo* studies investigating the role of phytate in impairing the bioavailabilities of minerals have been conducted with chicks, rats, mice, pigs and humans; some species differences among foodstuffs have also been reported (O'Dell et al., 1972; Reddy et al., 1989; Bobilya et al., 1991). Reddy et al. (1989) stated that the formation of insoluble phytate-mineral complexes in the intestinal tract of animals and humans prevents mineral absorption.

Supplementation of phytase to swine diets makes it plausible that the inorganic minerals mentioned previously are liberated and their bioavailabilities improved as a result of hydrolysis of the phosphate groups from the phytate molecules. Various *in vivo* experiments with pigs have been conducted to evaluate the effect of phytase supplementation on the bioavailabilities of macro and trace minerals (Näsi, 1990; Adeola, 1995; Adeola et al., 1995; Yi et al., 1996; Kemme, 1999b). It was shown that phytase is effective in increasing Ca digestibility and retention (Simons et al., 1990; Näsi, 1990; Young et al., 1993; Kemme et al., 1999b; Traylor et al., 2001). In addition, phytase also improves the absorption of Mg, Cu, Fe, and Zn (Näsi, 1990; Pallauf et al., 1992; Lei et al., 1993a; Adeola et al., 1995).

Effect of Phytase on Ca Bioavailability

Phytic acid and phytate residues in feedstuffs may interact with Ca and cause a reduction in its bioavailability to nonruminants (Campbell and Bedford, 1992). Although Ca has the lowest binding affinity *in vitro* (Maddaih et al., 1964; Vohra et al., 1965), the greatest impact of phytate on mineral nutrition other than P is on Ca bioavailability because it is the dominant mineral in most diets (Wise, 1983; Ravindran et al., 1995). High phytate intakes can reduce Ca absorption and its utilization for bone formation in rats, puppies, chickens and humans (Reddy et al., 1989). Oberleas et al. (1962) reported that phytic acid, added at 0.6 to 1.2% to the diet, caused a greater depression in growth rate in swine fed a high (1.5%) compared to a low (0.8%) Ca diet.

Supplementation of microbial phytase to swine diets significantly increased Ca digestibility and/or retention in many experiments (Jongbloed et al., 1993; 2000a).

Kemme and Jongbloed (1993) reported that the AFD of Ca was increased by 6 pu. Liu et al. (1997) showed that microbial phytase linearly increased the amount of Ca absorbed (g/day) ($P < 0.03$) and the percentage of Ca absorbed ($P < 0.08$) with increasing levels of supplementation (0, 250 and 500 FTU/kg diet) to a corn-soybean meal diet (low in P) fed to growing pigs. In agreement with results from this experiment are some other studies conducted by Simons et al. (1990), Näsi (1990), Young et al. (1993), Mroz et al. (1994), and Radcliffe et al. (1995). To a phytate-rich diet based on wheat, barley and soybean meal, Pallauf et al. (1994) reported a positive effect of phytase supplementation on the digestibility and retention of Ca in young piglets. Daily Ca excretion in feces of weanling pigs was reduced by 52% ($P < 0.0001$) upon phytase supplementation (750 FTU/kg diet) (Lei et al., 1993b). Li et al. (1998) reported that the AFD of Ca was also improved ($P < 0.05$) by supplementation of Phytase Novo® (750 FTU/kg diet). Eeckhout and De Paepe (1991) reported a highly positive correlation between Ca and P digestibilities and phytase supplementation to a diet low in P for swine (cited in Kornegay, 1999). Yi et al. (1996), however, found only small changes in the AFD of Ca upon phytase supplementation to a soybean meal based semipurified diet fed to young pigs (initial BW 7.5 ± 0.2 kg). Similar non-improvement results in the AFD of Ca were found by Adeola (1995) and Traylor et al. (2001).

Since the mid 1980's, many experiments were also carried out in The Netherlands, in which Natuphos® was supplemented to corn-soybean meal diets. In one experiment conducted with growing-finishing pigs, it was reported that the apparent Ca digestibility was related to its dietary content (4, 6, or 8 g/kg diet) and the rate of phytase supplementation (0, 300, or 600 FTU/kg diet). The maximal increase (10.8 pu) was obtained from the diet with the lowest Ca level and the highest rate of phytase supplementation (Jongbloed et al., 1993). Summarizing the six experiments (including the aforementioned one), which were tabulated in Jongbloed et al. (1993), one can conclude that the magnitude of increase in digestible Ca ranged from 0.14 to 0.79 g/kg when microbial phytase was supplemented at a rate from 330 to 1560 FTU/kg diet. Later, Kemme et al. (1999b) reported that the AFD of Ca was enhanced by 9.7 pu with Natuphos® supplementation (900 FTU/kg diet). A higher Ca digestibility, in practical diet formulation, implies that supplementation of Ca to diets can be slightly reduced in

the presence of microbial phytase, which may be beneficial as this lowers the buffering capacity of the diet (Jongbloed et al., 2000a). The supplementation of 500 FTU/kg diet generated 0.4 to 0.7 g/kg of digestible Ca, equivalent to 1.7 to 3.0 g limestone per kg of diet, assuming a 60% digestibility of Ca from limestone (Jongbloed et al., 2000a). Based on daily gain, digestible Ca and rib ash percentages in two trials, Kornegay et al. (1996) estimated Ca equivalency values of 1.08 and 0.38 g Ca, respectively, with an average of 0.73 g Ca, per 500 FTU of microbial phytase (cited in Kornegay, 1999).

Effect of Phytase on Zn Bioavailability

The deficiency of Zn was first discovered in 1955 in pigs fed a diet composed of natural plant products (corn and soybean meal). Thereafter, phytate-induced Zn deficiency was shown in rats, chicks, humans and pigs (Oberleas et al. 1962; Reddy et al., 1989; Campbell and Bedford, 1992; Szkudelski, 1995). Since Zn forms a highly insoluble salt at pH 6.0 (Maddaih et al., 1964), which is the approximate pH in the upper intestine of nonruminants where most absorption occurs, it can become deficient when a diet high in phytate is fed. Furthermore, the bioavailability of Zn can also be affected by the phytate-Ca synergism. The Zn-Ca-phytate complex, formed in the upper gastrointestinal tract, could render 80 to 90% of dietary Zn unavailable (Ravindran et al. 1995; Adeola et al., 1995).

Some information is available from the literature showing that phytase supplementation improved the apparent absorption of Zn. Pallauf et al. (1992) found that Natuphos® (500 to 1000 FTU/kg diet) increased the apparent Zn digestibility by 7 to 13 pu in piglets (9 to 25 kg BW) fed a corn-soybean meal diet containing 60 mg Zn/kg. Lantzsch and Wjst (1992) also reported that Natuphos® supplementation (1000 FTU/kg diet) to a diet containing 5 g Ca/kg increased the digestibility of Zn by 19 pu, as cited by Jongbloed et al. (1993). Pallauf et al. (1994) found that supplementation of Natuphos®, from 0 to 350, and then to 700 FTU/kg diet, enhanced ($P < 0.05$) the plasma Zn concentration from 7.79 to 9.48, and to 9.78 µmol/L, respectively. Similar positive effect of Finase™ (1350 FTU/kg diet) on the bioavailability of dietary Zn in weanling pigs (7.38 ± 0.37 kg initial BW) fed a corn-soybean meal diet were reported by Lei et al. (1993a). Ashida et al. (1999) then reported an improvement in Zn bioavailability in

growing pigs fed a corn-soybean meal diet supplemented with phytase. The results from a mineral balance study indicated that the daily Zn retention increased by 13.7 pu when Natuphos® (1500 FTU/kg diet) was supplemented to a corn-soybean meal diet (Adeola et al., 1995). Näsi and Helander (1994), however, found no improvement in Zn retention upon phytase supplementation to a barley-soybean meal diet fed to growing pigs, although the fecal excretion of Zn tended to be lower. The Zn equivalency value for phytase has not been established yet.

Effect of Phytase on the Bioavailabilities of Cu, Mg, Mn and Fe

The effect of phytase supplementation on the bioavailabilities of minerals other than P, Ca and Zn has received only limited attention in swine nutrition. Some information from the literature showed that Natuphos® improved the apparent absorption of Mg, Cu and Fe. Pallauf et al. (1992) reported that the apparent digestibilities of Mg, Cu and Fe increased by 8 to 13, 3 to 7, and 2 to 9 pu, respectively, while the absorption of Mn was not affected. Lantzsch and Wjst (1992) reported that Natuphos® supplementation (1000 FTU/kg diet) to a diet containing 5 g Ca/kg increased the digestibility of Mg by 7 pu, as cited by Jongbloed et al. (1993). Kemme et al. (1999b) reported that Natuphos® supplementation enhanced the AFD of Mg by 6.3 pu in growing-finishing pigs. Similar results were also reported by Windisch and Kirchgessner (1996). Adeola et al. (1995) found that the absorption and retention of Cu were increased ($P < 0.05$) when Natuphos® was supplemented to a corn-soybean meal diet (1500 FTU/kg diet), but the absorption and retention of Mg and Mn were not affected ($P > 0.05$). Similarly Pallauf et al. (1994) did not find improvement in Mg absorption. Stahl et al. (1999) reported that phytase hydrolyzed phytate effectively in corn-soybean diets and subsequently released phytate-bound Fe for hemoglobin repletion in young anemic pigs. The dose response curves for the effect of phytase on the digestibilities/bioavailabilities of Mg, Mn and Fe are not as clearly defined as for P (Kornegay, 1999).

E. Effect of Phytase on Nitrogen Utilization

Interactions of Phytate with Protein/AA

It has been widely accepted that the adverse effect of phytic acid/phytate in human diets is not only due to its interactions with essential minerals, but also due to its interactions with proteins (Cheryan, 1980; Reddy et al., 1989). More recently, the decreased utilization of N in the presence of phytic acid/phytate in animal diets has also been realized to have nutritional and economic implications. In theory, there are totally four mechanisms that can explain the lower digestibilities of dietary protein and AA with relation to phytic acid/phytate: 1) phytate-protein/AA complexes inherent in feedstuffs, 2) *de novo* formation of phytate-protein complexes in the digestive tract, 3) *de novo* formation of phytate-free AA complexes after digestion of dietary protein in the digestive tract, and 4) formation of the complexes involving phytate and proteolytic enzymes in the gastrointestinal system (Selle et al., 2000; Kies et al., 2001).

Several earlier studies with human foods, including cereal grains and oilseed meals, during 1950's already reported that phytate interacts with some proteins to form insoluble products or complexes (O'Dell and De Boland, 1976). According to Hartman (1979), there are about 2 to 3% of protein that are strongly complexed to phytate in the commercial soy protein isolates. Microstructural and chemical evidence demonstrated phytic acid to be located in the protein bodies of the soybean, likely in the form of a soluble protein-phytate salt. The probable existence of a protein-mineral-phytate complex *in situ* was shown by gel filtration in which Ca and P was found to coelute with soluble proteins (Prattley and Stanley, 1982). A recent work done by Jongbloed (1997) and his colleagues, however, showed that it is unlikely that intrinsic phytate-protein complexes exist in several plant feedsuffs to a extent that is quantitatively important in their three fractions of soluble protein. Whether this is also the case for insoluble protein remains to be elucidated (Jongbloed, 1997; Kies et al., 1997). However, it is generally assumed that inherent phytate-protein complexes, which is less accessible to proteolytic enzymes during intestinal transit, are present in the feedstuffs of plant origin for nonruminant animals (Ravindran et al., 1995; Sebastian et al., 1998).

In the digestive tract of pigs, phytic acid/phytate can *de novo* bind dietary protein and free AA at the certain pH that normally occur *in situ*, forming insoluble complexes of phytate-protein/AA or phytate-mineral-protein/AA (Honig and Wolf, 1991; Szkudelski, 1995, 1997) (Figure I-1). Under acidic conditions, the basic phosphate groups of phytic acid may complex with amino groups such as lysyl, histidyl and arginine. Under neutral conditions, the carboxyl groups of some amino acids may bind to phytate through a divalent or trivalent mineral (Cheryan, 1980; Kornegay, 2001). Obviously, these bindings can render protein less susceptible to proteolysis, and therefore, decrease the digestibilities of CP and individual AA (Reddy et al., 1989; Szkudelski, 1997; Kemme et al., 1999a). *In vitro* studies carried out by Knuckles et al. (1985, 1989) showed that phytate and its hydrolysates reduced the digestion of casein and bovine serum albumin by 7 to 14% and 1 to 7%, respectively. A recent *in vitro* study (Jongbloed, 1997) showed that a very strong complex between soluble protein and phytate was formed at pH 2 to 3 in several feedstuffs of plant origin. Pre-incubation of phytate with phytase will prevent the formation of such a complex. If the complex has been formed, protein can be liberated from the phytate by pepsin, but this process can be accelerated considerably by phytase addition. Another *in vitro* study carried out by Rutherfurd et al. (1997) showed that phytate could bind free AA. After incubation of rice pollards (which is rich in phytate) with lysine-HCl, approximately 20% of free lysine was bound. However, half of this amount was liberated after addition of phytase (2000 FTU/kg diet). *In vivo* studies with rats and fish showed that phytate could reduce ($P < 0.05$) the efficiency of dietary utilization of N (Atwal et al., 1980; Caldwell, 1992); however, the results of *in vivo* studies are not always consistent due to the complicated interactions between phytate and protein within animal body (Thompson and Serraino, 1986; Knuckles et al., 1989).

Another explanation for the decrease in protein digestion by phytate is the inhibition of proteolytic enzymes, such as trypsin and pepsin. Phytate could inhibit digestive enzymes via direct binding of enzymatic proteins or by indirect chelation of cations that are cofactors for enzyme function (Reddy et al., 1989; Caldwell, 1992; Szkudelski, 1997). Under *in vitro* conditions, it was observed that trypsin activity was substantially inhibited by phytate at different concentrations (10 to 100 mM), especially at 37°C (Singh and Krikorian, 1982). In theory, this inhibition could render the enzyme

less effective in the digestive tract, an increase in endogenous protein/AA losses, and therefore, a decrease in protein/AA digestibilities.

To summarize almost all of the previous investigations regarding the interactions between phytate and protein/AA, it can be concluded that the factors affecting the formation of phytate-protein/AA complexes include the type of protein, the solubility rate of protein, *in situ* pH, contents of other dietary minerals, and the three way interactions between phytate, proteolytic enzymes, and protein/AA (Kemme et al., 1999a). Therefore, to predict the extent to which phytate-protein complexes exist and how they affect digestibilities of minerals and protein/AA is very complicated.

Phytase, as was mentioned before, is an enzyme that can catalyze the chemical degradation of phytic acid or phytate, i.e. the cleavage of phytate-protein/AA or phytate-mineral-protein/AA bonds. The *in vivo* mechanisms of phytase on protein digestion and/or AA absorption are not clear yet. However, it is assumed that phytate-protein/AA and/or phytate-mineral-protein/AA complexes in the gastrointestinal tract of animals are hydrolyzed stepwise by phytase and, therefore, more protein/AA are released for digestion and absorption (Jongbloed et al., 2000a). Selle et al. (2000), however, concluded that the rationale for the protein responses to microbial phytase remains largely speculative, and several modes of action are probably involved. As yet, the effect of phytase supplementation to diets for pigs on N digestibility and retention has given results varied from none to small improvements in the literature.

Effect of Phytase on Fecal CP/AA Digestibilities and N Retention

The effect of phytase supplementation on the AFD of dietary protein and AA has been measured in several studies with pigs. Although the AFD of CP is commonly accepted as an inaccurate measurement of protein bioavailability (Sauer and Ozimek, 1986), under practical conditions, it is important to the determination of the environmental N burden in response to phytase supplementation (Jongbloed et al., 2000a). Khan and Cole (1993) found that the CP digestibility was increased by 4.1 pu (from 81.4 to 85.5%) upon supplementation of microbial phytase (1000 FTU/kg diet) (cited in Jongbloed et al., 2000a). Ketaren et al. (1993) reported that the addition of microbial phytase to a sucrose-soybean meal diet (1000 FTU/kg) for growing pigs

increased not only the ratio of protein retained to protein intake (0.33 vs. 0.36 kg/kg; $P < 0.05$), but also the daily protein deposition in pigs (108 vs. 123 g/d; $P < 0.05$). Studies also with growing pigs (45 to 110 kg BW) fed a corn-tapioca-soybean meal-barley-peas diet by Mroz et al. (1994) showed that supplementation of Natuphos® (800 FTU/kg diet) increased the CP digestibility by 2.3 pu (from 83.3 to 85.6%, $P < 0.01$). Of the indispensable AA, the digestibilities were improved ($P < 0.01$) from 1.4 pu for tryptophan to 2.8 pu for threonine. In addition, N retention increased from 40.1 to 42.8%, albeit not significantly ($P > 0.05$). On the basis of average daily gain (**ADG**) and feed efficiency, Biehl and Baker (1996) concluded that AA utilization in young pigs (5 to 20 kg BW) was improved upon phytase supplementation (1200 FTU/kg) to corn-soybean meal diets deficient in AA. Han et al. (1997) reported that microbial phytase supplementation (1000 to 1200 FTU/kg diet) resulted in a higher apparent digestibility of CP by 3.0 pu (from 79.9 to 82.9%) and N retention by 2.4 pu (from 71.9 to 74.3%) in pigs from weaning to finishing fed a corn-soybean meal diet. However, the differences were not significant ($P > 0.05$). Improvements in the AFD of N ($P < 0.05$) were also reported by Wenk et al. (1993) with growing pigs (25 to 100 kg BW). Based on results summarized from the literature, Jongbloed et al. (2000a) concluded that supplementation of phytase increased the apparent N digestibility by 0.60 ± 1.66 pu (n = 10).

In contrast to the positive responses to phytase supplementation on the AFD of N or CP, other studies did not show a significant effect (Näsi et al., 1990; Ketaren et al., 1993; Näsi and Helander, 1994; Pallauf et al. 1994; Valaja et al., 1998; Li et al., 1998). Näsi et al. (1995) showed only a marginal improvement in the AFD of CP (from 78.0 to 78.8%) in the presence of Finase® FP500 (1000 FTU/kg diet), whereas there was a slight decrease in N retention (from 40.6 to 39.4%). Fecal CP digestibility data, however, cannot be used to entirely exclude a positive effect of phytase on protein bioavailability because of the effect of bacterial fermentation on the protein and AA entering the large intestine (Sauer and Ozimek, 1986).

Effect of Phytase on Ileal CP/AA Digestibilities

The ileal digestibility values of dietary protein and AA are considered better estimates of their bioavailabilities than fecal digestibilities (Sauer and Ozimek, 1986;

Sauer et al., 2000). However, only a limited number of studies have been carried out to determine the effect of phytase supplementation on ileal AA digestibilities. Though a few reports show that microbial phytase exerted no effect on the ileal digestibilities of CP and AA (Valaja et al., 1998), the majority of studies have shown that supplementation of microbial phytase to swine diets increased the AID of CP and most AA. The improvements were usually significant or at least there was a trend. The magnitude of the overall improvement was about 1 to 2 pu based on a conservative estimate (Kies et al., 1997).

Officer and Batterham (1992) reported that supplementation of microbial phytase to a semi-synthetic diet containing 40% Linola™ meal for growing pigs (40 kg BW) increased the CP digestibility by 7 to 12 pu; the digestibilities of AA increased by 4 to 13 pu. However, only the increases in lysine and histidine were significant ($P < 0.05$) (Table I-2). Khan and Cole (1993) reported an increase in N digestibility by 12.8 pu in gilts fed a high phytate barley-based diet upon microbial phytase supplementation (1000 FTU/kg diet). Mroz et al. (1994) reported a smaller effect of *Aspergillus niger* phytase supplemented to a corn-tapioca-soybean meal-barley-peas diet (800 FTU/kg) fed to growing pigs. The AID of arginine and methionine increased by 2.5 and 3.9 pu, respectively ($P < 0.01$). Numerical increases by 1.2 to 3.6 pu ($P > 0.05$) were also found in this study for the AID of CP and some AA (Table I-2). Kornegay et al. (1998) reported that the AID of CP and most AA increased linearly ($P < 0.10$ to 0.001) when phytase was added (0, 250 and 500 FTU/kg) to a low CP corn-soybean meal diet (Table I-2). In the trial of Kemme et al. (1999a), in which Natuphos® was supplemented to a corn-soybean meal diet (900 FTU/kg diet) for growing-finishing pigs (37 to 95 kg BW), there was a trend towards improvement in the AID of CP (by 1.6 pu, $P = 0.056$). The AID of lysine, tryptophan, threonine and isoleucine improved ($P < 0.05$) by 2.4, 4.4, 2.9 and 2.1 pu, respectively; there was also a trend towards improvements in the AID of arginine and phenylalanine ($P < 0.10$) (Table I-2). Recently, Traylor et al. (2001) reported that the apparent and true ileal digestibilities of CP and most AA increased slightly upon phytase supplementation at a rate of 500 FTU/kg diet, but not at higher rates (1000 to 1500 FTU/kg diet); in most cases there was a cubic effect ($P < 0.05$) (Table I-2).

The results obtained by Officer and Batterham (1992), Mroz et al. (1994), and Kemme et al. (1999a) showed that supplementation of Natuphos® (500 to 1000 FTU/kg diet) increased the AID of CP and usually of most AA (Table I-2). The increases were significant ($P < 0.05$) in some instances. Using the studies of Mroz et al. (1994) and Kemme et al. (1999a) as the basis and assuming a linear response to phytase, the additional amounts of apparent ileal digestible CP and some indispensable AA resulting from phytase supplementation were calculated by Kies et al. in 1997 (Table I-3). The additional amounts of ileal digestible indispensable AA ranged from 0.02 for tryptophan to 0.10 g/kg diet for lysine. Also using a linear regression approach, and based on 8 trials with growing/finishing pigs and one trial with sows, Kies et al. (2001) updated their previous calculations (Kies et al., 1997) for the additional amounts of ileal digestible CP and indispensable AA resulting from phytase supplementation (500 FTU/kg diet). In general, the new values were in the same range as those calculated previously (Table I-3).

In contrast to the aforementioned results, Lei et al. (1997) and Valaja et al. (1998) reported no effect of phytase supplementation on ileal CP and AA digestibilities. The discrepancy may possibly arise from the type of diets employed and the feeding regimen. Different diets provide different sources and levels of protein and AA, and especially different levels of phytate. The large effect obtained by Officer and Batterham (1992) might be because of Linola™, which is a raw linseed material with moderate quality after oil separation. The digestibilities of CP and AA of Linola™ meal without phytase supplementation were only around 50 to 60% (Table I-2). The non-positive results of Valaja et al. (1998) are not surprising since they used "wet barley protein with fiber", a by-product from an integrated starch-ethanol process, as the only source of dietary protein. This protein source hardly contains any phytate and already has a relatively high CP digestibility, approximately 80%.

F. Effect of Phytase on Energy Utilization and Other Parameters

Effect of Phytase on Energy Utilization

Phytic acid and phytate residues in nonruminant diets may decrease starch digestibility by one or more of the following mechanisms: 1) by complexing dietary starch directly and/or indirectly through a protein linkage, as shown in Figure I-1, 2) by inhibiting α-amylase and/or β-galactosidase through direct binding these enzymes, and/or 3) by indirectly chelating minerals such as Ca, which are needed for carbohydrases activation (Graf, 1986; Reddy et al., 1989; Szkudelski, 1997; Kornegay, 1999). An inhibitory effect of phytate on lipase activity was also found in an *in vitro* experiment (Knuckels, 1988). Supplementation of microbial phytase to swine diets may neutralize these antinutritional effects, thereby increasing the digestibilities of starch, lipids and protein, and therefore, the contents of digestible energy (**DE**) and/or metabolizable energy (**ME**).

A number of studies with pigs have been performed to determine the effect of phytase supplementation on the utilization of energy. Officer and Batterham (1992) reported that the DE content of a diet containing Linola™ meal was increased from 12.2 to 12.7 MJ/kg upon *Aspergillus niger* phytase supplementation. A marked increase ($P < 0.05$) in energy digestibility upon phytase supplementation (1000 FTU/kg diet) was also reported by Wenk et al. (1993) in studies with growing-finishing pigs fed diets containing barley, corn, dried potatoes, soybean meal, and fish meal. Ketaren et al. (1993) observed an increase in the ratio of energy retained to DE intake after phytase supplementation to a diet (1000 FTU/kg) for growing gilts (0.38 vs. 0.36 MJ/MJ, $P < 0.05$). Khan and Cole (1993) found that upon phytase supplementation (1000 FTU/kg diet), the AFD of gross energy (**GE**) was increased by 1.2 pu; however, the difference was not significant. The increased energy value derived from the additional digestible protein is equivalent to 21.6 kJ NE/kg diet, 32.5 kJ DE/kg diet, or 30.5 kJ ME/kg diet, when phytase was included into the diet at a rate of 500 FTU/kg. However, this calculation was only based on a limited number of experiments with pigs (Kies et al., 2001).

Effect of Phytase on Other Parameters

During some studies with pigs on the mineral- and/or protein-related effects of phytase, its effects on the improvements of other parameters, such as the digestibilities of dry matter (**DM**), organic matter, ash, crude fiber, crude carbohydrates, and/or ether extract, were also determined, mainly in passing. In theory, any change in digestion and/or absorption of dietary protein, carbohydrates, or lipids will affect organic matter digestibility; any change in mineral absorption will affect ash digestibility, while DM digestibility depends on both organic matter and ash digestibilities. Kemme et al. (1999b) reported an enhancement of ash AFD by 7.3 pu (52.2 to 59.5%, $P < 0.001$) upon Natuphos® supplementation (900 FTU/kg diet). Similar effects were also reported earlier by Näsi and Helander (1994), and Näsi et al. (1995). For organic matter, Mroz et al. (1994) reported an improvement of AFD from 85.6 to 87.2% ($P < 0.01$). For DM, they reported an improvement of AFD from 83.2 to 85.0% ($P < 0.01$). Similar effects were also reported by Officer and Batterham (1992), Beers and Jongbloed (1992a), and Li et al. (1998). For ether extract, improvements ($P < 0.05$) were reported by Näsi et al. (1995) and Valaja et al. (1998). Meanwhile, there are also many studies (e.g., Simons et al., 1990), in which there were no improvements ($P > 0.05$) in the digestibilities of the aforementioned parameters.

G. Effect of Phytase on Performance

The effect of phytase supplementation on swine performance has been evaluated in many studies. The evaluation parameters used among investigators are quite diverse, including ADG, average daily feed intake (**ADFI**), feed conversion ratio (**FCR**), carcass composition, and bone mineralization. The results obtained from different experiments are not always consistent.

Simons et al. (1990) found that the growth rate and FCR of pigs fed diets low in P supplemented with microbial phytase were similar to or better than those obtained from pigs fed the control diets. Cromwell (1991) reported improvements ($P < 0.05$) in ADG, FCR, and bone strength in growing-finishing pigs fed a diet supplemented with an

unpurified preparation from *Aspergillus niger* (500 or 1000 FTU/kg diet). Higher ADG and ADFI ($P < 0.001$), and better FCR ($P < 0.01$) were also reported by Beers and Jongbloed (1992a) in piglets fed a corn-barley-soybean meal diet supplemented with microbial phytase (1450 FTU/kg). Ketaren et al. (1993) reported that the addition of Natuphos® to a grower diet (1000 FTU/kg) increased the ADG (741 vs. 835 g/d, $P < 0.05$), and improved the FCR (2.37 vs. 2.16, $P < 0.01$). In studies by Cromwell et al. (1993) with growing-finishing pigs, the addition of microbial phytase (Finase™) to a basal diet (250, 500 or 1000 FTU/kg) resulted in linear increases in ADG, ADFI, FCR, and bone strength ($P < 0.01$ or 0.05). Lei et al. (1993b) and Young et al. (1993) also supplemented Finase™ (500 to 1350 FTU/kg diet) to diets for piglets; improvements were reported for ADG ($P < 0.07$), ADFI ($P < 0.01$) and FCR ($P < 0.01$). Peter et al. (2001) showed that supplemental levels of inorganic P, Zn, Cu, and Mn can be reduced or completely eliminated from corn-soybean meal diets fed to pigs during the late finishing stage with Natuphos® supplementation (300 or 500 FTU/kg diet). There were no deleterious effect on growth performance and carcass characteristics of the pigs (84 to 123 kg BW). In agreement with these effects on pig performance are results obtained by Kornegay and Qian (1996), Adeola et al. (1995), Li et al. (1998), and Grandhi (2001). The relative ratios between the diet supplemented with phytase and the diet without phytase for growth rate, feed intake and FCR were about 106, 103, and 95.7, respectively, suggesting additional positive effects of microbial phytase on swine performance (Jongbloed et al., 2000a). For piglet performance, the results cited from the literature and expressed as deviations in growth-corrected feed : gain ratios (i.e. FCR) are presented in Figure I-4, which shows that the feed : gain ratio improves with increasing rates of microbial phytase supplementation (Kies et al., 1997, 2001).

On the other hand, as cited by Jongbloed et al. (1993), Kessler and Egli (1992) found no differences ($P > 0.05$) in the performance of growing-finishing pigs (24 to 107 kg BW) after supplementation of Finase™ (500 FTU/kg diet). Growth performance, protein and energy deposition in the body, as well as carcass and bone characteristics, were not affected upon phytase supplementation in the study by Fandrejewski et al. (1999).

The improvement in animal performance, however, should not be surprising in many cases, since the effect of phytase supplementation may be larger than can be attributed to P only. The improvements in bioavailabilities of other minerals, proteins, AA, and starch may all contribute to the improvement in animal performance. One can conclude that phytase can maintain or improve pig performance even in the absence of inorganic P supplementation, if phytase is supplemented according to recommendations (Lei et al., 1993c; Khan, 1995; Han et al., 1997).

H. Factors Affecting the Efficacy of Microbial Phytase

Utilization of phytic acid/phytate by nonruminants is a very complicated enzymatic process and, therefore, the magnitude of the response to supplemental microbial phytase is affected not only by the level of supplementation, but also by the physiological status of the pig, and many other dietary and management factors. Dietary factors include the levels of phytate, some nutrients (inorganic P, Ca, other minerals, vitamin D, etc.), and mineral chelators. Management factors include feed processing, feeding regimen, and housing conditions. The intrinsic phytase activity in feed ingredients may also play a role in affecting the response of the animal to added phytase. Understanding of these factors, which interact or govern the animal response to phytase, will help the feed industry and pig producers in managing the use of this enzyme.

The intricate and complex events that occur *in vivo* are nearly impossible to be duplicated via *in vitro* studies. Therefore, the *in vitro* results obtained cannot be simply applied to the *in vivo* conditions. Even the *in vivo* results obtained under experimental conditions may not always be applicable to the practical conditions, in which animals are fed more complex or different diets. The factors affecting the efficacy of microbial phytase may be responsible for some of the contradictory results in the literature, although different results among various studies may demonstrate a lack of standardization in experimental procedures (Khan, 1995).

Excess dietary Ca not only precipitates phytate progressively by forming insoluble phytate-Ca complexes, but also interacts with soluble phytate, thereby reducing the susceptibility of phytate to enzymatic hydrolysis. Skoglund et al. (1997) found that phytate hydrolysis in the colon of pigs was impaired by addition of calcium carbonate to a barley-rapeseed cake-peas diet (12.5g/kg). An excess of Ca could also directly decrease phytase activity by competing for the active sites of the enzyme (Wise, 1983; Pointillart et al., 1985; Qian et al., 1996; Kornegay, 1999). Therefore, the absolute Ca content in the diet is of importance in determining the extent of intestinal hydrolysis of phytate (Sebastian et al., 1998). Lei et al. (1994) reported that even at a normal dietary Ca concentration (0.8%), an adverse effect on the efficacy of supplemental phytase was detected. Düngelhoef and Rodehutscord (1995) reported in a review that high Ca levels reduce the absorption of P and the utilization of phytate by pigs. Li et al. (1999), in studies with cannulated pigs (45 kg BW) fed corn-soybean meal diets, concluded that the efficacy of microbial phytase to hydrolyze phytate-P appeared to increase when the dietary level of Ca was reduced from 0.8 to 0.4%. Seynaeve et al. (2000a) reported that addition of limestone had little effect on the digestibilities of OM, P and Ca, but reduced the activity of supplemental phytase and had a negative influence on growth performance. In short, the higher the dietary Ca content, the lower the efficacy of phytase (Pointillart et al., 1989; Lei et al., 1994; Lantzsch et al., 1995; Adeola et al., 1998; Jongbloed et al., 2000a). Use of chelators, such as citrate which can remove Ca from soluble phytate complexes, is effective *in vitro* in increasing the activity of phytase (Maenz et al., 1999).

Wide dietary Ca : P ratios have been reported to have adverse effects on phytase activity *in vivo*. The depressive effect of Ca on phytase activity was greater at the lower dietary P level, which suggests that phytase activity can be influenced by the dietary P level or Ca : P ratio (Seynaeve et al., 2000b). When the dietary P level exceeds the requirement of the pig, there will only be a very small effect on pig performance (Jongbloed, 1987). Based on results from other studies, as stated by Qian et al. (1996), when the ratio of Ca : P exceeded 2.0 : 1, there were usually adverse effects on performance, bone characteristics and serum criteria of pigs. However, no significant effects were observed when the ratio was below 2.0 : 1, especially in the range of 1.0 : 1

to 1.6 : 1. Qian et al. (1996) also found that narrowing the Ca : P ratio from 2.0 : 1 to 1.2 : 1 led to an approximate 16% increase in the efficacy of phytase for improving digestibility, performance, bone measurements, and serum Ca levels. Liu et al. (1996, 1998) reported that pig performance (ADG, FCR and bone strength) and P utilization (P absorption and bone ash weight) were increased ($P < 0.001$ to 0.08) by lowering the Ca : P ratio from 1.5 : 1 to 1.0 : 1 in corn-soybean meal diets (low in P) supplemented with microbial phytase. In most previous studies, in which phytase was supplemented to diets low in P, the Ca : P ratios were high because Ca was supplemented to obtain a level similar or close to NRC (1988) standards. For example, the low P corn-soybean meal diets fed to pigs during the growing phase in studies by Cromwell et al. (1993, 1995) had Ca : P ratios ranging from 1.9 : 1 to 2.3 : 1. In the study by Lei et al. (1994) to determine the dietary Ca level on the efficacy of phytase, the calculated ratios were approximately 1.6 : 1 and 3.3 : 1. Calculations by Jongbloed et al. (1995) showed that for maximal P retention, at a marginal supply of P, the optimum ratio of digestible Ca to DP ranged from 1.2 : 1 to 2.3 : 1. In summary, the efficacy of phytase may be enhanced in a low P diet by reducing Ca supplementation below NRC (1988) standards to lower the Ca : P ratio. The suggested Ca : P ratio for cereal-soybean meal diets is between 1.0 : 1 to 1.25 : 1; the suggested ratio based on available P is between 2.0 : 1 to 3.0 : 1 (NRC, 1998).

Vitamin D has been shown to affect the utilization of phytate-P (Wise, 1983; Adeola et al., 1998). Supplementation of a diet containing 0.6% P, 80% phytate-P, and 0.6% Ca with cholecalciferol (vitamin D_3) at 1,000 IU/kg nearly doubled the absorption and retention of P in pigs, although the activities of intestinal phytase and alkaline phosphatase were not affected (Fontaine et al., 1985). The formation of unavailable Ca-phytate complexes took place with time in vitamin D-depleted pigs (Pointillart et al., 1985). Therefore, the effect of dietary Ca on hydrolysis of phytate-P may be counteracted by a high level of vitamin D, which promotes Ca absorption and thus prevents or at least reduces the formation of Ca-phytate complexes (Pointillart et al., 1989). Using weanling pigs fed corn-soybean meal diets, Lei et al. (1994) reported that the inclusion of a high level of vitamin D (6,660 vs. 660 IU/kg) in diets with normal Ca contents (0.8%) may partially offset the adverse effect of Ca, but the phytase efficacy was not enhanced in diets low in Ca (0.4%). Biehl and Baker (1996) reported that the

addition of 1α-hydroxycholecalciferol (1α-OH D_3) to a P deficient corn-soybean meal diet was not effective in improving phytate-P utilization in pigs. However, 1α-OH D_3 is effective in poultry for reasons yet unknown. Li et al. (1998) also found that there was no increase in phytase efficacy ($P > 0.05$) as a result of addition of vitamin D_3. Interactions between phytase and Ca or vitamin D may have somewhat confounded the effect of phytase (Lei et al., 1994).

The amount and source of phytate and/or intrinsic phytase activity in swine diets are other factors influencing the supplemental phytase efficacy. It was observed that a level of phytate-P at 1.8 g/kg generated substantially more DP in a diet mainly containing corn, while in a diet mainly containing sunflower seed meal only slightly more DP was generated. Therefore, it was concluded that the lower level (1.2 g/kg) of phytate-P in both diets was too low to obtain the maximal effect of the enzyme. And it was also concluded that phytate in corn is more readily available than phytate in sunflower seed meal (Jongbloed et al., 2000a). Lack of substrate (i.e. phytate) may often occur in piglet diets formulated with large proportions of animal products as protein or P source, or of plant ingredients that are high in intrinsic phytase activity such as wheat, wheat bran, barley, rye or triticale (Jongbloed et al., 2000a).

Minerals, pH, and Mineral Chelators

Minerals can readily bind to phytic acid and thus have the potential to form insoluble mineral-phytate precipitates and soluble mineral-phytate complexes that are resistant to the hydrolysis by phytase. This hypothesis is consistent with the incomplete utilization of dietary phytate-P and the negative effect of minerals on phytate-P digestibility. The effects of minerals, pH, and mineral chelators on the susceptibility of phytate to hydrolysis by microbial phytase (Natuphos®) were investigated by Maenz et al. (1999) using a simple solution and a slurry of canola meal under defined *in vitro* conditions. The results showed that in a simple solution, mineral concentrations from 0.053 mM for Zn^{2+} up to 4.87 mM for Mg^{2+} caused a 50% inhibition of phytate-P hydrolysis by microbial phytase at pH 7.0. The potency-rank order of the minerals as inhibitors of phytate hydrolysis was $Zn^{2+} >> Fe^{2+} > Mn^{2+} > Fe^{3+} > Ca^{2+} > Mg^{2+}$ at neutral pH. Acidification of the media to pH 4.0 decreased the inhibitory potency of all the

divalent cations tested. The inhibitory potency of Fe^{3+} showed a moderate increase with declining pH. Inclusion of 25 mM ethylenediamine-tetraacetic acid (**EDTA**) completely blocked Ca^{2+} inhibition of phytate hydrolysis at pH 7.0. In a slurry of canola meal, supplementation with mineral chelators such as EDTA, citrate and phthalate increased the efficacy of microbial phytase for hydrolysis of phytic acid. Inclusion of 100 mM phthalic acid plus phytase in the canola meal slurry resulted in complete hydrolysis of phytate-P. Competitive chelation by compounds such as EDTA, citric acid or phthalic acid has the potential to decrease enzyme-resistant forms of phytic acid and thereby improve the efficacy of microbial phytase.

Feed Processing and Diet Acidification

Feed processing can affect the interactions between phytate and nutrients, and therefore, the efficacy of the supplementary phytase (Ravindran et al., 1995). The high temperature during pelleting, expansion, and extrusion of feedstuffs can reduce the microbial load and may increase nutrient bioavailability. On the other hand, depending on the processing conditions, there can also be reductions in the availabilities of carbohydrates, proteins and trace elements, and inactivation of vitamins. Phytase, a protein, like many other enzymes, can be easily inactivated during pelleting. It did appear that steam-pelleting at temperature higher than 60°C strongly reduce phytase activity (Nunes, 1993). When the temperature of steam-pelleting reaches approximately 80°C, the absorption of P and Ca in pigs can be decreased by 10 pu or more, which is presumably due to the reduction in intrinsic phytase activity (Jongbloed and Kemme, 1990). By pelleting a pig diet at 70°C, the initial activity of supplemental microbial phytase was reduced by 15 to 25% (Jongbloed et al., 1993, 2000a).

Soaking a phytate-rich diet supplemented with microbial phytase (500 FTU/kg diet) for 8 to 15 h before feeding resulted in an additional enhancing effect on P and Ca digestibilities, namely by 8 and 6 pu, respectively (Kemme and Jongbloed, 1993). Soaking of a diet for rats supplemented with phytase increased the absorption of Mg, Fe, Zn, Mn, Ca and P (Szkudelski, 1995). Soaking a pig diet (formulated with barley, peas and rapeseed cake, without phytase supplementation) at room temperature for 9 h before feeding resulted in a 45% reduction in phytate content and a 3-fold increase in the amount

of free P, which may be due to the activation, by moisture, of the intrinsic phytase present in the cereals (Skoglund et al., 1997). Further reduction in the fecal phytate content was demonstrated in pigs fed the diet both soaked with whey (40°C, 3 h, feed : water = 1 : 1) and supplemented with microbial phytase at 1000 FTU/kg diet (Skoglund et al., 1998). Näsi and Helander (1994), on the other hand, found no response on mineral availability after 3-h soaking of a barley-soybean meal diet supplemented with phytase (1200 FTU/kg) in studies with growing pigs. However, in another experiment, Näsi et al. (1995) reported that soaking a barley-rapeseed meal diet for 3 h with dried whey in water at 40°C increased the apparent absorption of P by 3 pu. Liu et al. (1997) reported that soaking for 2 h at 30°C increased the efficacy of microbial phytase supplemented to a corn-soybean meal diet. There were no differences in P digestibility and growth performance ($P > 0.05$) when phytase was supplemented at a rate of 250 FTU/kg diet with soaking and at a rate of 500 FTU/kg diet without soaking.

It can be hypothesized that wet soaking of diets before feeding may cause partial hydrolysis of phytate. The practice of wet feeding of growing-finishing pigs is reported to have increased, especially in Europe, and has the potential of enhancing phytase efficacy and improving P bioavailability (Skoglund et al., 1997). Reports from the industry also show that phytase is more efficient under these feeding systems (Kornegay, 1999). However, in the opinion of the authors, soaking of diets may not be convenient under practical feeding conditions.

Dietary acidification may have a synergistic effect with microbial phytase to enhance the efficacy of phytase. This means that the dietary inclusion of organic acids (such as acetic acid, citric acid, formic acid or lactic acid) in combination with phytase may improve the digestibilities of minerals and AA (Jongbloed et al., 2000a). Supplementation of a corn-soybean meal diet with *Aspergillus niger* phytase (900 FTU/kg diet) and simultaneous acidification with lactic acid (30 g/kg) resulted in a greater increase in AFD of P than was calculated as the sum of the stimulatory effects of the single additions (Kemme et al., 1999b). A synergistic effect of organic acids and microbial phytase was also found for the AFD of P, Mg and ash, but not for pig performance (Jongbloed et al., 2000b). In agreement with this study are results obtained by Valencia and Chavez (1997), Han et al. (1998), and Li et al. (1998). Chang and

Chiang (1997) reported that supplementation of fumaric acid to diets had no effect on the efficacy of phytase in further improving P digestibility in pigs. Radcliffe et al. (1998) and Boling et al. (2000) also reported no synergistic effect between citric acid and microbial phytase on pig performance and the digestibilities of some of the nutrients tested. Kemme et al. (1999a) also reported no synergistic effect between lactic acid and phytase on ileal AA digestibilities in a study with growing-finishing pigs. At present, the interaction between phytase and organic acids remains unclear. To understand the relationship between diet acidification and phytase efficacy, further research is warranted (Kornegay, 1999).

Physiological Status

Physiological status in terms of age, body weight, or categories of pigs all seem to influence the hydrolysis of dietary phytate and the efficacy of microbial phytase. However, information on these aspects is scarce and the results are not always consistent (Jongbloed et al., 1993; Kornegay, 1999). With respect to categories of pigs, Kemme et al. (1997a) reported that phytase efficacy decreased in the following order: lactating sows > growing-finishing pigs > sows at the end of pregnancy > piglets > sows at mid-pregnancy. The efficacy of phytase was particularly low in sows on day 60 of pregnancy, high in lactating sows, and intermediate in growing-finishing pigs (Kemme et al., 1997c). The difference among categories of pigs is probably related to differences in gastric retention times. However, the requirement for DP by different categories of pigs should also be taken into account (Kemme et al., 1997a, c). Harper et al. (1997) reported that in one trial the apparent digestibilities for P and Ca were slightly higher during the grower than during the finisher phase, but no difference was found in the second trial. The efficacy of microbial phytase was compared between pigs of 16 and 39 kg BW fed a semi-purified diet low in P by Rodehutscord et al. (1998), and there was no difference in P digestibility (cited in Kornegay, 1999). It seems that there is no indication that the efficacy of microbial phytase is influenced by the body weight of pigs (Kornegay, 1999).

Feeding Regimens

In vitro studies indicated that hydrolysis of phytic acid and/or phytate in feedstuffs is related to the incubation time. Although homogeneous mixing of the enzyme with the respective diet influences the efficacy of phytase, feeding regimens, such as level and frequency of feeding, may also affect its efficacy (Khan, 1995). Mroz et al. (1994) reported that the level of feeding (2.3 vs. 2.8 times the maintenance requirement for ME) had a minor influence on the efficacy of phytase in growing-finishing pigs fed a corn-tapioca-soybean meal diet. When fed at 2.8 times the maintenance requirement for ME, there was a small decrease in the efficacy of phytase, which may be related to a faster rate of gastric emptying and rate of passage of digesta. In the same experiment, there was no difference in phytase efficacy when the pigs were fed 2 or 7 times daily; however, feeding only once daily reduced the AFD of Ca, tryptophan, and isoleucine, and also the AID of phytic acid, cystine, arginine, isoleucine and phenylalanine ($P < 0.05$). Other management factors such as housing conditions may also affect the digestibility values or the efficacy of microbial phytase in a lesser extent (Kemme et al., 1997b; Jongbloed et al., 2000a)

I. Phytase Research in the Future

In consequence of the research on microbial phytase over the past ten years, the implication of this enzyme to the swine industry is obvious to date. In addition to reducing the phosphate output, the use of phytase may allow for more cost-effective formulation of diets owing to its positive effects on mineral, protein/AA, and energy utilization. However, in light of current knowledge, it seems not possible to exceed P availabilities of 60 to 70% in feed ingredients of plant origin, even if phytase is supplemented at a very high rate. The results of phytase effects on the utilization of other nutrients are not consistent at present. All these indicate that there are some key areas present in phytase application, remaining to be investigated. First of all, the *in vivo* mechanisms of phytate and phytase actions on different dietary nutrients should be further clarified. Secondly, certain dietary and even management conditions, which are

required for the biochemical hydrolysis of phytate in pigs fed practical diets (Weremko et al., 1997; Maenz et al., 1999), need to be determined.

Desirable phytase should have high catalytic activity, broad substrate specificity, high thermo-stability, good resistance to proteolysis, high residual activity within a pH range from 2.5 to at least 7.7, and ease of use. Presently, one of the problems facing the feed industry is the loss of phytase activity at processing temperatures exceeding 80°C. Therefore, the compatibility of commercial phytase preparations with the conditions imposed by feed processing and diet feeding should be improved (Lei and Stahl, 2001; Zyla, 2001). Fortunately, research is underway to develop a more heat-stable phytase that can withstand the high temperature during pelleting (Newman, 1991; Wyss et al., 1999a, b; Kies et al., 2001). The search for new desirable phytases is also being actively pursued at present time (Wodzinski and Ullah, 1996; Matsui et al., 2000; Stahl et al., 2000; Lei and Stahl, 2001).

Although further research is warranted for efficient use of phytase, its use in the swine industry is expected to be expanded in the future due to the serious environmental impact of P pollution, which may lead to legislation in more and more countries who wish to deal with animal manure in a nutrient management plan. Selection of a phytase product depends not only on its efficacy, but also on its economic benefits. In the near future, much attention will also be paid to the effects of phytase on nutrients other than P, and its economic equivalence more than just an alternative to inorganic phosphate. Presently, at the beginning of a new millennium, one of the challenges for swine nutritionists and producers is to maximize and secure all the known beneficial effects that arise from phytase application. One immediate effort, however, should be made to determine the optimal dietary and management conditions for microbial phytase to act efficiently and economically.

J. Objectives of This Research Project

The general objective of my PhD research project was to determine the effect of phytase supplementation to different diets fed to weanling and growing pigs on the

utilization of various nutrients, including ash, DM, and energy. Particulars are as follows:

1. To study the effect of phytase supplementation to four different diets fed to weanling pigs on the utilization of various macro- and micro-minerals, including ash and DM.
2. To study the effect of phytase supplementation to four different diets fed to weanling pigs on the utilization of CP, AA, and energy.
3. To study the effect of phytase supplementation to a high- and to a low-phytate diet fed to growing pigs on the utilization of various macro- and micro-minerals, including ash and DM.
4. To study the effect of phytase supplementation to a high- and to a low-phytate diet fed to growing pigs on the utilization of CP, AA, and energy.

Table I–1. Contents of phytic acid and phytate phosphorus, and activities of intrinsic phytase of some feed ingredients used for swine[a]

Ingredients	Phytic Acid (%)[b]	Phytate P (%)	Phytate P (% of total P)	Intrinsic Phytase Activity (FTU/kg)[c]	Literature Sources[d]
Cereal grains					
Corn	0.75	0.24	70.9	29 (0-56)	C, E, K, N, P, R, R4, R5, T, W
Barley	0.85	0.23	61.2	529 (200-882)	C, E, M, N, P, R, R5, T, W
Wheat	0.93	0.26	67.5	1118 (300-2000)	C, E, K, M, N, P, R, R5, T, W
Wheat bran	3.35	0.81	71.9	2848 (600-5208)	E, K, M, N, P, R, R4, R5, T
Oats	0.98	0.26	64.0	42 (0-108)	C, E, M, N, R, R5
Rye	0.78	0.22	61.0	3456 (1782-6128)	E, R, W
Rye bran	2.13	0.60	60.0	5150 (2300-8000)	P
Triticale	0.99	0.27	68.5	1428 (1200-2039)	E, P, R, T
Rice	0.78	0.23	76.5	--	D, M, R, R4, R5, T
Rice bran	3.95	1.07	74.3	96 (0-145)	E, K, N, R, R4, R5, T
Sorghum (Milo)	0.81	0.22	70.8	24 (0-76)	E, N, R, R4, R5, T
Millet	0.59	0.17	64.4	--	R, R4, R5,
High protein feeds					
Soybean meal	1.40	0.37	56.7	28 (0-188)	C, D, E, K, N, P, R, R4, R5, T
Canola (Rapeseed) meal	3.38	0.63	58.2	16 (0-36)	E, K, M, P, R5, W
Peas	0.86	0.23	58.5	116 (36-183)	E, M, R, R4, R5
Cottonseed meal	2.92	0.82	73.3	--	C, N, R5, T
Peanut meal	1.94	0.46	63.5	3 (0-8)	E, M, P, R, R5
Flax meal	4.20	0.69	56.0	23 (0-41)	E, R
Sunflower meal	2.73	0.69	63.0	62 (0-185)	E, M, P, R5, T
Corn gluten meal	1.28	0.36	58.8	48 (0-177)	C, E, K, N, R5
Sesame meal	3.60	1.03	81.0	--	C, N, R5
Miscellaneous					
Alfalfa meal	0.04	0.01	4.0	60 (15-250)	C, E, N, R5
Coconut meal	1.31	0.27	46.3	24 (0-80)	E, M, R4, R5

[a] The average values, expressed on an air dry basis, were calculated from different numbers of literature sources.

[b] The contents of phytic acid were usually calculated by most investigators on the assumption that phytic acid contains 28.2% phosphorus.

[c] Values in parentheses indicate the range in intrinsic phytase activity. One FTU is defined as that amount of phytase that liberates 1 mmol of ortho-phosphate per minute from 5.1 mM Na-phytate at pH 5.5 and 37°C (Eeckhout and De Paepe, 1994; Engelen et al., 1994, 2001).

[d] C = Cromwell (1992); D = de Boland et al. (1975); E = Eeckhout and de Paepe (1994); K = Kirby et al. (1988); M = Maga (1982); N = Nelson et al. (1968); P = Pointillart (1988); R = Reddy et al. (1989); R4 = Ravindran et al. (1994); R5 = Ravindran et al. (1995); T = Tyagi et al. (1998); W=Weremko et al. (1997).

Table I–2. Effect of microbial phytase supplementation to swine diets on apparent ileal digestibilities (%) of crude protein and amino acids (AA)

Phytase added	Officer and Batterham (1992)		Mroz et al. (1994)		Kornegay et al. (1998)		Kemme et al. (1999)		Traylor et al. (2001)	
(FTU/kg diet)	0	1000	0	800	0	500	0	900	0	500
Crude protein	53[a]	65[b]	71.7	74.2	66.7	70.1	74.2	75.8	82.5	83.4[d]
Indispensable AA										
Arginine	-	-	84.6[a]	87.1[b]	84.0	85.9[c]	83.8	85.3	93.2	93.7
Histidine	57[a]	69[b]	76.3	77.7	80.4	81.7	81.4	82.1	89.4	89.8
Isoleucine	65	72	80.1	79.8	73.0	75.9[c]	77.9[a]	80.0[b]	87.9	88.8
Leucine	64	72	82.0	81.7	79.9	81.6	83.8	85.1	86.4	87.6
Lysine	59[a]	71[b]	81.0	81.9	72.7	76.0	77.5[a]	79.9[b]	89.9	90.7[d]
Methionine	71	75	76.7[a]	80.6[b]	75.8	78.0	80.6	81.7	90.2	90.6
Phenylalanine	67	74	81.6	81.3	78.6	80.8[c]	80.0	81.7	82.5	83.4[d]
Threonine	50	62	73.8	72.0	66.0	70.0[c]	68.3[a]	71.2[b]	80.5	81.3
Tryptophan	-	-	72.4	73.6	-	-	68.3[a]	72.7[b]	89.3	90.9[d]
Valine	63	70	78.7	78.4	70.0	73.5[c]	76.1	78.1	85.8	86.6
Dispensable AA										
Alanine	-	-	-	-	71.4	74.3[c]	78.3[a]	80.2[b]	84.0	84.6
Aspartic acid	-	-	-	-	74.4	77.6[c]	75.2[a]	77.7[b]	87.4	88.1
Cystine	68	81	70.5	74.1	74.7	77.8[c]	73.4	73.0	82.2	82.7
Glutamic acid	-	-	-	-	82.3	84.7[c]	84.1	85.6	91.5	92.1
Glycine	-	-	-	-	62.9	68.7[c]	63.7[a]	67.2[b]	77.5	79.4
Proline	-	-	67.6	70.6	78.9	81.0	81.6	82.6	83.8	84.9
Serine	-	-	-	-	78.0	80.4	78.1	79.8	86.0	87.0[d]
Tyrosine	63	69	-	-	73.8	76.4	79.7[a]	81.8[b]	85.6	86.8[d]

[a, b] Digestibility values in the same row within each study with different superscripts differ ($P < 0.05$ or 0.01).

[c] Linear effect of phytase was found with supplementation rates at 0, 250, and 500 FTU/kg diet ($P < 0.10$ to 0.001).

[d] Cubic effect of phytase was found with supplementation rates at 0, 500, 1000, and 1500 FTU/kg diet ($P < 0.05$ to 0.01).

Table I–3 Additional amounts (g/kg diet)[a] of ileal digestible crude protein and indispensable amino acids liberated by phytase supplementation (500 FTU/kg diet)

Items	Kies et al. (1997)[b]	Kies et al. (2001)[c]
Crude protein	3.0	1.953
Amino Acids		
Arginine	--	0.088
Histidine	--	0.015
Isoleucine	0.05	0.040
Leucine	--	0.114
Lysine	0.10	0.075
Methionine	0.04	0.026
Cystine	0.03	0.024
Phenylalanine	--	0.059
Threonine	0.04	0.054
Tryptophan	0.02	0.030
Valine	--	0.037

[a] Average of the values obtained directly from reports or indirectly from linear regression.

[b] Based on 2 trials with growing/finishing pigs.

[c] Based on 8 trials with growing/finishing pigs and one with sows.

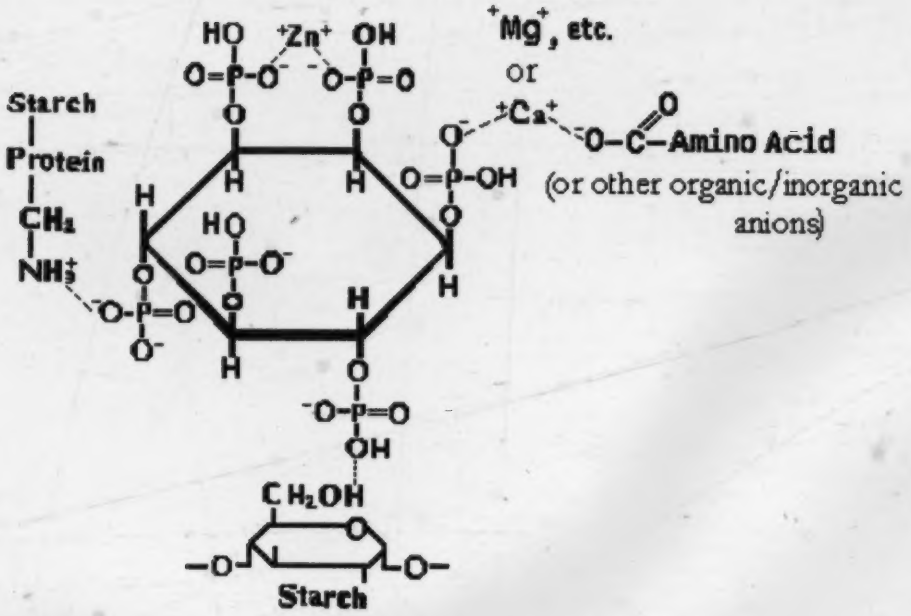

Figure I–1. A model of a phytate molecule and possible interactions with some nutrients (modified after Thompson, 1986)

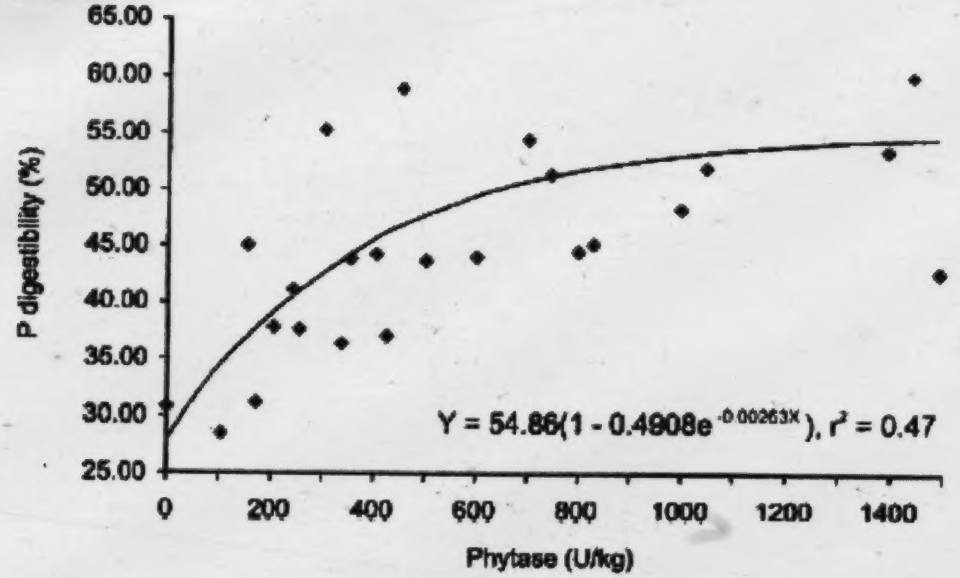

Figure I–2. Phosphorus digestibility of pigs fed low P, low phytase activity plant-based diets supplemented with microbial phytase (Kornegay et al., 1998; Kornegay, 1999)

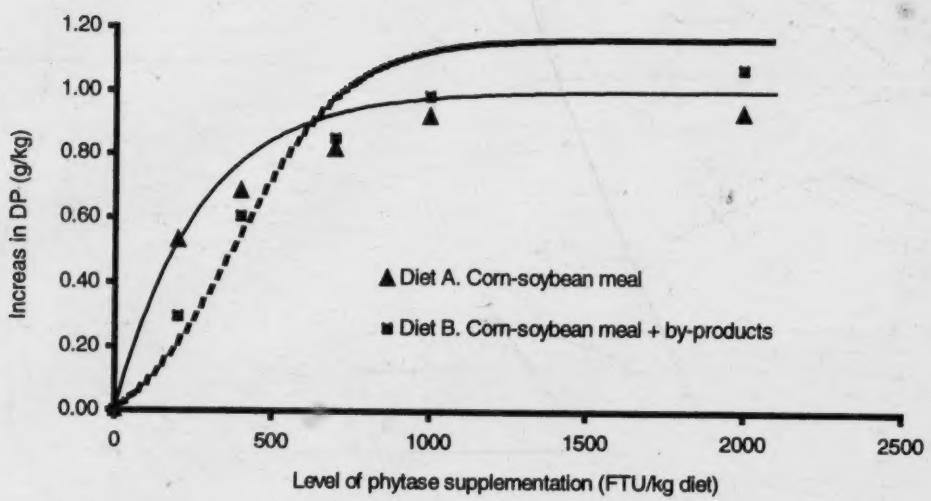

Figure I-3. The effect of increasing levels of microbial phytase on the contents of digestible phosphorus (DP) in growing pigs (Beers and Jongbloed, 1992b).

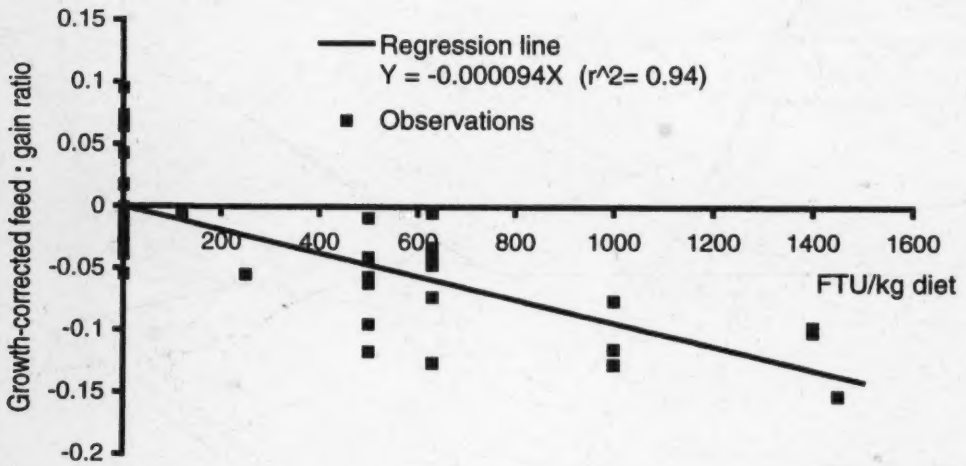

Figure I–4. Effect of microbial phytase (Natuphos®) supplementation on the relative performance of piglets (compared to control) fed diets not limiting in available phosphorus (Kies et al., 2001).

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CHAPTER II. EFFECT OF PHYTASE SUPPLEMENTATION ON THE UTILIZATION OF SELECTED MINERALS IN DIFFERENT DIETS FED TO WEANLING PIGS [1]

A. Introduction

Abundant in many feed ingredients of plant origin, phytate/phytic acid is one of the most important anti-nutritional factors to nonruminant animals. The very limited ability of pigs to utilize phytate-phosphorus (P) poses at least three problems to swine producers, feed manufacturers, environmentalists, and the general public as well. The first problem involves the supplementation of inorganic P, which is expensive, to swine diets. Sometimes, inorganic P is over supplied to ensure that the requirement for bioavailable P is met. The second problem is environmental P pollution resulting from the excretion of a large proportion of P in swine manure. Growing concerns over this issue have been globally expressed, especially in Europe and North America. The third problem relates to the ability of phytate/phytic acid to form complexes with other dietary nutrients including minerals, proteins, free amino acids, and starch (Liao et al., 2002). Phytase is an enzyme that catalyzes the cleaving of orthophosphate groups from phytate molecules. However, pigs lack this enzyme.

Numerous investigations have shown that supplementation of microbial phytase to swine diets improves the digestibility and retention of P (Simons et al., 1990; Cromwell et al., 1993; Lei et al., 1993b, c). The optimum supplementation rate appears to be 500 FTU/kg diet, although a maximum response can be achieved at 1,000 FTU/kg diet (Khan, 1995; Jongbloed et al., 2000). However, nearly all studies were carried out with corn-soybean meal diets without inorganic P supplementation. Therefore, further studies on

[1] Portions of this study were presented at 2003 Banff Pork Seminar, Banff, AB, Canada, and the 9th International Symposium on Digestive Physiology in Pigs, 2003, Banff, AB, Canada.

other diets with inorganic P supplementation, as in practice, are also warranted. The effect of phytase supplementation on the digestibilities and/or retention of other minerals, such as calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), and copper (Cu) have been investigated only in a few studies, and the results were not always consistent (Pallauf et al., 1992, 1994; Adeola, 1995; Adeola et al., 1995; Kemme et al., 1999). Therefore, further studies on these minerals were also needed.

It was hypothesized that the efficacy of microbial phytase varies with different dietary compositions, while the supplementary level of phytase may influence the actual animal response. The present study was carried out to investigate the effect of phytase supplementation to different starter diets on the digestibilities of P and some selected minerals, namely Ca, Mg, Fe, Mn, Cu, and Zn, in weanling pigs. Mineral balance studies were also carried out for P and Ca. A further objective of this study was to determine if a higher level of phytase supplementation (1,000 vs. 500 FTU/kg diet) can further improve the mineral bioavailabilities in different diets.

B. Experimental Procedures

Experiments and Diets

Four experiments were carried out with weanling pigs. In each experiment, the piglets were fed a basal diet consisting of ingredients commonly used in western-Canada (Table II-1). To each basal diet, *Aspergillus niger* phytase (Natuphos®, DSM Food Specialties, Delft, The Netherlands) was supplemented at rates of 500 and 1,000 phytase units (**FTU**) per kilogram to formulate two more experimental diets for each of the four experiments. One FTU is defined as the quantity of enzyme that liberates 1 mmol of ortho-phosphate per minute from 5.1 mM Na-phytate at pH 5.5 and 37°C (Engelen et al., 2001). The diets were supplemented with inorganic P (a mixture of mono- and di-calcium phosphate) to meet the NRC (1998) standards for available P, which is 0.32% for weanling pigs. Canola oil was included in the diets to reduce the dustiness and to increase the digestible energy content up to the level recommended by NRC (1998). The calculated metabolizable energy (ME) contents, based on NRC (1998), of the basal diets

used in Exp. 1, 2, 3 and 4 were 3,183, 3,240, 3,248 and 3,302 kcal/kg, respectively. Free AA were supplemented, when necessary, to meet the NRC (1998) standards on the basis of their apparent ileal digestible supply. Vitamins and minerals were supplemented to meet or exceed NRC (1998) standards. Chromic oxide was included in the diet at a rate of 0.30% as the digestibility indicator.

As shown in Table II-1, the major ingredients of the basal diet in Exp. 1 were corn and soybean meal. In Exp. 2, the major ingredients were wheat and soybean meal. In Exp. 3, the major ingredients were wheat, soybean meal and canola meal. In Exp. 4, the major ingredients were barley, peas and canola meal. The major ingredients were ground through a 2-mm mesh screen prior to diet formulation. Prior to surgery and during the recuperation period, the piglets were fed, *ad libitum*, a starter diet containing 20% CP. All diets were fed in the form of mash. Water was freely available from a low-pressure drinking nipple.

Animal Trial Procedures

For each of the four experiments, six PIC barrows (Camborough × Canabrid), weaned at three weeks of age, were obtained from the University of Alberta Swine Research Unit. The barrows were housed individually in metabolism crates (height: 82 cm; length: 124 cm; width: 76 cm) in a barn, in which the temperature was maintained between 25 and 28°C. On d 6 and 7 after weaning and adjustment to the crates, each piglet was fitted with a simple T-cannula at the distal ileum, approximately 5 cm from the ileo-cecal sphincter. Detailed descriptions of cannula preparation, surgery, pre- and post-operative care were previously given by Sauer (1976) and Li et al. (1993). Intact pigs are usually used for the study of fecal digestibilities of minerals. However, in this trial cannulated pigs were used for reasons that these pigs were also used for determination of the ileal digestibilities of CP and AA (Chapter III). It needs to be pointed out here that the cannulation does not affect fecal digestibility values (Sauer and Ozimek, 1986).

Following a 1-wk recuperation period after surgery, the piglets were fed three experimental diets according to a repeated 3 × 3 Latin square design. Each experimental period comprised 14 d. For Exp. 1 and 2, the diets were fed to the piglets at a rate of 2.4 times the maintenance requirement for ME (i.e., 100 kcal/kg of $BW^{0.75}$), based on the

individual BW of each piglet which was determined at the beginning of each experimental period (Table II-3). For Exp. 3 and 4, the diets were fed to the piglets at a rate of 5% of BW (equivalent to 2.7, 2.9, and 3.1 times the maintenance requirement for ME in periods 1, 2, and 3 respectively). The daily meal allowances were offered twice daily at 0800 and 2000, equal amounts each meal.

The experimental proposal was reviewed and approved by the Faculty of Agriculture, Forestry and Home Economics Animal Care Committee of the University of Alberta in accordance with the guidelines of CCAC (1993).

Sample Collection and Chemical Analysis

Samples of the major feed ingredients were taken prior to diet formulation. Samples of the diets were taken during the time the meal allowances were prepared. The collection of feces and urine was initiated at 0800 on d 8 of each experimental period and continued for 96 consecutive hours. Feces were collected by aid of colostomy bags (Stomahesive® Wafer, Sur-Fit® Natura™, ConvaTec, Princeton, NJ, USA). The bags were changed every 4 to 8 h depending on the amount of feces collected. Feces were frozen at –28°C immediately after collection. Urine, collected through glass wool, were stored at –4°C immediately after collection, then filtered through triple layers of medical gauze and pooled for each pig and frozen at –28°C. Ileal digesta were collected from 0800 to 2000 on d 12, 13 and 14. The contents of minerals in ileal digesta were not determined. The digesta were solely collected to determine the ileal amino acid digestibilities of the diets as was reported in Chapter III.

Prior to chemical analysis, feces were air-dried and then pooled, leaving one sub-sample for each pig in each experimental period. All samples including those of ingredients and diets, were ground through a 0.5-mm mesh screen in a Thomas-Wiley Laboratory Mill (Arther H. Thomas Co., Philadelphia, PA, USA). Urine samples for mineral analysis were first filtered with Whatman #2 filter paper, and then air-dried at 60°C.

Dry matter was measured according to AOAC International (2000) official method 930.15. Ash was measured according to AOAC International (2000) official method 942.05. Gross energy was determined with an AC-300 Leco Automatic Calorimeter

(Leco® Corporation, St. Joseph, MI, USA). Crude protein (N × 6.25) was measured with a Leco FP-428 Nitrogen Determinator. Total P content was determined photometrically by the molybdovanadate procedure according to AOAC International (2000) official method 965.17. The phytate P contents in the basal diets were analyzed according to the procedure described by Haug and Lantzsch (1983). Calcium, Mg, Fe, Mn, Cu and Zn were analyzed with an atomic absorption spectrophotometric procedure according to AOAC International (2000) official method 968.08. For Ca determination, lanthanum chloride was included at the final dilution step providing 1% (g/ml) lanthanum to minimize interference from other minerals. The intrinsic phytase activities (FTU/kg diet) were analyzed with a colorimetric enzymatic procedure according to AOAC International official method 2000.12 (Engelen et al., 1994, 2001). Chromic oxide was determined with a spectrophotometric procedure according to Fenton and Fenton (1979). Analyses of ingredients and diets were carried out in triplicate; analyses of urine and feces in duplicate.

Digestibility Calculations and Statistical Analysis

The apparent fecal digestibilities (**AFD**) of the aforementioned minerals, ash and DM were determined. In addition, the urinary excretions of P and Ca were also determined in order to determine their retentions. The apparent digestibilities were calculated by using the following equation:

$$D_D = 100\% - [(A_F \times I_D) / (A_D \times I_F)] \times 100\%$$

where D_D is the apparent digestibility of a nutrient in the assay diet (%); A_F is the nutrient concentration in feces (%); I_D is the indicator concentration in the assay diet (%); A_D is the nutrient concentration in the assay diet (%); I_F is the indicator concentration in feces (%).

Based on the following linear model, the digestibility and balance values were subjected to statistical analysis by using the General Linear Model (GLM) Procedure of SAS® (1990).

$$Y_{ijk} = \mu + T_i + P_j + A_k + \varepsilon_{ijk}$$

where Y_{ijk} is a digestibility or balance value; μ is the overall mean of the digestibility or balance values; T_i is the fixed effect of treatments (i.e. phytase

supplementation) and $i = 1, 2, 3$; P_j is the random effect of experiment periods and $j = 1, 2, 3$; A_k is the random effect of animals and $k = 1, 2, 3, 4, 5, 6$; ε_{ijk} is the random experimental error with N $(0, \sigma^2)$. After analysis of variance (ANOVA), means of treatments were compared by using the Student-Newman-Keuls multiple range test. Probability levels of $P \leq 0.05$ and $0.05 < P \leq 0.10$ were defined as significant differences and tendencies, respectively.

C. Results

Diet Analysis and Animal Health

The chemical compositions of the basal diets are presented in Table II-2. The analyzed values of the nutrients in the diets were close to the values calculated based on the analyzed values in the ingredients. The phytate-P contents of the four basal diets ranged between 0.16 and 0.22% (as-fed basis), while the intrinsic phytase activities ranged between 53 and 419 FTU/kg. The content of CP in diet 4 was 18.9% and lower than in the other diets which ranged from 20.3 to 21.3%. Diet 4 included free methionine, threonine, and tryptophan (Table II-1).

All piglets remained healthy and usually consumed their meal allowances within 30 min after feeding throughout the experiments. The average BW at the initiation of the experiments, at the beginning of each experimental period, and the conclusion of the experiments are presented in Table II-3. The ADG of the pigs in Exp. 1, 2, 3 and 4 were 178, 221, 216 and 211 g/d, respectively. Postmortem examinations, conducted at the conclusion of each experiment, revealed no adhesions or any other intestinal abnormalities.

Phosphorus Balance Study

Phytase supplementation to the basal diets improved the AFD of P ($P < 0.001$) in all experiments (Table II-4). The magnitudes of improvement were 6.5 to 9.5 percentage units (**pu**) with the corn-soybean meal diet (Exp. 1), 9.1 to 12.7 pu with the wheat-

soybean meal diet (Exp. 2), 8.5 to 11.2 pu with the wheat-soybean meal-canola meal diet (Exp. 3), and 11.8 to 11.9 pu with the barley-peas-canola meal diet (Exp. 4). Except with the wheat-soybean meal diet (Exp. 2), there were no differences ($P > 0.10$) in the AFD of P between the two rates of phytase supplementation (500 vs. 1,000 FTU/kg). With the wheat-soybean meal diet (Exp. 2), the AFD of P was higher ($P < 0.05$), namely by 3.6 pu, when phytase was supplemented at a rate of 1,000 FTU/kg.

The daily output of P in feces was decreased with phytase supplementation at both rates ($P < 0.05$ to 0.001), except with the wheat-soybean meal-canola meal diet (Exp. 3) in which supplementation at 500 FTU/kg only tended ($P < 0.10$) to decrease the fecal output of P (Table II-4). The fecal output of P when phytase was supplemented at a rate of 1,000 FTU/kg was lower ($P < 0.05$) than when phytase was supplemented at a rate of 500 FTU/kg in Exp. 1 and 2, but not ($P > 0.05$) in Exp. 3 and 4. Phytase supplementation decreased the fecal output of P by 13.8% (9.0 to 18.7%) with the corn-soybean meal diet (Exp. 1), 25.4% (20.7 to 30.2%) with the wheat-soybean meal diet (Exp. 2), 15.8% (10.9 to 20.8%) with the wheat-soybean meal-canola meal diet (Exp. 3), and 27.3% (26.5 to 28.2%) with the barley-peas-canola meal diet (Exp. 4), on the average of both rates.

The daily urinary output of P tended to increase ($P < 0.10$) upon phytase supplementation to the diets in Exp. 1, 2 and 3, but not the diet in Exp. 4 ($P > 0.10$) (Table II-4). The retention of P was increased ($P < 0.05$ to 0.001) upon phytase supplementation. The magnitudes of increase were 5.9 to 8.1 pu (12.7 to 17.5%) with the corn-soybean meal diet, 8.7 to 11.1 pu (15.6 to 19.9%) with the wheat-soybean meal diet, 8.4 to 10.1 pu (21.3 to 25.6%) with the wheat-soybean meal-canola meal diet, and 11.3 to 11.5 pu (20.9 to 21.3%) with the barley-peas-canola meal diet in Exp. 1, 2, 3 and 4, respectively. There were no differences ($P > 0.05$) in P retention between the two rates of phytase supplementation in all experiments (Table II-4).

Calcium Balance Study

The effect of phytase supplementation on the AFD of Ca varied with the composition of diets (Table II-5). Supplementation of phytase increased the AFD of Ca in the piglets fed the wheat-soybean meal diet (Exp. 2) ($P < 0.01$) and the barley-peas-canola meal diet (Exp. 4) ($P < 0.05$). The magnitudes of improvement ($P < 0.05$) were

8.7 to 8.8 pu with the wheat-soybean meal diet (Exp. 2), and 8.6 to 8.9 pu with the barley-peas-canola meal diet (Exp. 4). There was no effect ($P > 0.10$) of phytase supplementation on the AFD of Ca in the piglets fed the corn-soybean meal diet (Exp. 1) and the wheat-soybean meal-canola meal diet (Exp. 3). As well, in all experiments there were no differences ($P > 0.10$) in the AFD of Ca between the two rates of phytase supplementation.

The fecal output of Ca was decreased only significantly ($P < 0.05$) in Exp. 2 after phytase supplementation to the wheat-soybean meal diet (Table II-5). There were no differences ($P > 0.10$) in the output of Ca between the two rates of phytase supplementation. In Exp. 4, although the F test showed that phytase supplementation to the barley-peas-canola meal diet had a significant effect ($P = 0.043$) on the fecal output of Ca, the Student-Newman-Keuls multiple range test showed that there was only a tendency ($P < 0.10$) towards a decrease in Ca output. Phytase supplementation to the corn-soybean meal diet (Exp. 1) and the wheat-soybean meal-canola meal diet (Exp. 3) did not affect ($P > 0.10$) the daily output of Ca in feces.

There were no differences ($P > 0.10$) in the daily urinary output of Ca upon phytase supplementation in all experiments (Table II-5). Phytase supplementation to the wheat-soybean meal diet (Exp. 2) increased ($P < 0.01$) the retention of Ca, but not to the corn-soybean meal diet (Exp. 1) and the wheat-soybean meal-canola meal diet (Exp. 3). With the wheat-soybean meal diet (Exp. 2), there was no difference ($P > 0.10$) in the Ca retention between the two rates of phytase supplementation. With the barley-peas-canola meal diet (Exp. 4), the F test showed that the phytase supplementation had a significant effect ($P = 0.045$) on Ca retention, but the Student-Newman-Keuls multiple range test showed that there was only a tendency ($P < 0.10$) towards an increase in the retention.

Digestibilities of Other Minerals

The AFD of Mg, Fe, Mn, Cu, and Zn are presented in Table II-6. There was no effect ($P > 0.10$) of phytase supplementation to the four basal diets on the AFD of these minerals. There were numerical increases ($P > 0.10$) in the AFD of Mg, Mn, Cu, and Zn upon phytase supplementation, but not in the AFD of Fe.

Digestibilities of Ash and Dry Matter

The supplementation of phytase to the corn-soybean meal diet (Exp. 1) did not affect ($P > 0.10$) the AFD of ash (Table II-7). However, phytase supplementation to other three basal diets (Exp. 2, 3 and 4) increased ($P < 0.05$) the AFD of ash. The magnitudes of improvement were 4.9 to 6.4 pu for the wheat-soybean meal diet (Exp. 2), 3.6 to 4.0 pu for the wheat-soybean meal-canola meal diet (Exp. 3), and 7.4 to 8.0 pu for the barley-peas-canola meal diet (Exp. 4). There were no differences in the AFD of ash between the two rates of phytase supplementation ($P > 0.05$).

For the AFD of DM (Table II-7), the increases approached significance ($P < 0.10$) for the wheat-soybean meal diet (Exp. 2) and the barley-peas-canola meal diet (Exp. 4), but not ($P > 0.10$) for the corn-soybean meal diet (Exp. 1) and the wheat-soybean meal-canola meal diet (Exp. 3).

D. Discussion

Commercial preparations of microbial phytase were initially developed to increase the bioavailability of phytate-P in feedstuffs of plant origin for nonruminants in order to reduce the animal reliance on inorganic P supplementation, decrease the P output in manure, and alleviate P pollution to the environment. Previous investigations, mainly with corn-soybean meal diets fed to growing and/or finishing pigs without inorganic P supplementation, have shown that supplementation of microbial phytase can achieve the aforementioned goals (Simons et al., 1990; Näsi, 1990; Jongbloed et al., 1992; Cromwell et al., 1993, 1995; Mroz et al., 1994; Kemme et al., 1999). In this study, the supplementation of phytase to four practical-type diets for weanling pigs improved the AFD of P by 6.5 to 12.7 pu and rentention by 5.9 to 11.5 pu (Table II-4). Other studies with weanling pigs which were fed corn-soybean meal diets have also shown that the AFD of P was increased upon phytase supplementation (Beers and Jongbloed, 1992; Lei et al., 1993b; Young et al., 1993). The improvements in the AFD of P were larger in the aforementioned studies than in this study. The smaller improvements in this study can be explained by the relatively high concentration of total P in the diets (0.59 to 0.76%, Table

II-3) as inorganic P was supplemented. Lei et al. (1993b) fed a low-P corn-soybean meal diet that contained only 0.32% total P. Phytase supplementation improved the AFD of P from 46.4 to 69.0%. On the assumption that P from inorganic source (e.g., mono-dicalcium phosphate) is completely available (NRC, 1998), the improvements in AFD of P will be 11.5, 15.6, 12.9, and 19.0 pu in in Exp. 1, 2, 3, and 4, respectively. These improvements are more in the range of values reported for weanling pigs by Beers and Jongbloed (1992), Lei et al. (1993b), and Young et al. (1993).

The daily output of P in feces was reduced by 13.8, 15.8, 25.4, and 27.3% in Exp. 1, 3, 2 and 4, respectively (Table II-4). Daily output of P in urine was very small compared to feces, irrespective of phytase supplementation. Therefore, with an increase in AFD of P upon phytase supplementation there was a corresponding increase in P retention. This observation is in agreement with studies reported by Lei et al. (1993b). The results of this study imply that the standard for available P as suggested by NRC (1998), namely 0.32%, may be too low.

Phytic acid and phytate residues in animal diets may bind polyvalent cations, such as Ca^{2+}, Zn^{2+}, Cu^{2+}, Mg^{2+}, Mn^{2+}, and $Fe^{2+/3+}$, forming insoluble phytate-mineral complexes, rendering these minerals poorly available to nonruminants (Wise, 1983; Adeola et al, 1995; Szkudelski, 1995; Jongbloed et al., 2000). Reddy et al. (1989) stated that the formation of insoluble phytate-mineral complexes in the intestinal tract of animals and humans prevents mineral absorption. Supplementation of phytase to swine diets makes it plausible that these minerals are liberated and their bioavailabilities improved as a result of hydrolysis of the orthophosphate groups from the phytate molecules. Results reported in the literature regarding the effect of phytase supplementation on the the AFD of the aforementioned minerals were not consistent (Näsi, 1990; Pallauf et al., 1992; Lei et al., 1993a; Näsi and Helander, 1994; Adeola et al., 1995; Ashida et al. 1999).

Supplementation of phytase increased ($P < 0.05$) the AFD of Ca in Exp. 2 and 4, but not in Exp. 1 and 3. The retention of Ca was increased but this was significant only in Exp. 2 (Table II-5). These results show that a positive response to phytase supplementation occurred when the diets are relatively low in Ca. The dietary Ca levels were 0.84 and 0.70% in Exp. 2 and 4, respectively, and 0.94 and 1.05 in Exp. 1 and 3,

respectively (Table II-3). Most studies in which corn-soybean meal diets (without supplementation of P and/or Ca) were fed to growing and/or finishing pigs have shown that phytase supplementation increases the digestibility and/or retention of Ca (Näsi, 1990; Simons et al., 1990; Kemme and Jongbloed, 1993; Mroz et al., 1994; Liu et al., 1997). Studies with weanling pigs fed corn-soybean meal diets have also shown that phytase supplementation improves the AFD and/or retention of Ca (Lei et al., 1993b; Young et al., 1993; Pallauf et al., 1994; Radcliffe et al., 1995). On the other hand, studies have also been reported that showed no effect of phytase supplementation on the AFD of Ca and/or retention (Adeola, 1995; Yi et al., 1996; Traylor et al., 2001). With an experiment conducted with growing-finishing pigs, it was reported that the AFD of Ca was not only related to the rate of phytase supplementation (0, 300, or 600 FTU/kg) but also to the dietary Ca content (4, 6, or 8 g/kg). The largest increase (10.8 pu) was obtained from the diet with the lowest Ca content and the highest rate of phytase supplementation (cited in Jongbloed et al., 1993).

The AFD of Mg, Cu, Mn, Fe, and Zn were also determined in this study (Table II-6). It should be stressed that the sources of these minerals include both the feed ingredients and the inorganic supplements (Table II-1). There was no effect ($P > 0.10$) of phytase suplementation on the AFD of these minerals. The digestibilities of Cu (in most instances) and Zn were negative. As was stated previously, phytic acid and phytate residues may bind polyvalent cations, forming insoluble phytate-mineral complexes. Whether or not a particular phytate salt is formed depends on the pH, the phytic acid level, the presence of other cations, and the concentration of the particular mineral (Liao et al., 2002). Dietary Ca, at levels equal to or above the requirement of the animal, may reduce the absorption of Mg, Cu, and Zn, in the presence of dietary phytic acid (Adeola, 1995). The possible synergistic effect of two or more cations which may co-precipitate to increase the quantity of phytate-mineral complexes has been known (Maga, 1982; Ravindran et al., 1995); however, the *in vivo* mechanism by which phytate affects mineral utilization is not completely understood yet (Reddy et al., 1989). The relative stabilities of various phytate-mineral complexes were studied *in vitro* with potentiometric titration methods. Vohra et al. (1965) indicated a descending order of formation of phytate-mineral complexes at pH 7.4 as follows: $Cu^{2+} > Zn^{2+} > Ni^{2+} > Co^{2+} > Mn^{2+} > Fe^{3+} > Ca^{2+}$.

Maddaih et al. (1964) found the descending order of salt stability to be: $Zn^{2+} > Cu^{2+} > Co^{2+} > Mn^{2+} > Ca^{2+}$ at physiological pH. Zinc and Cu appear to have the highest affinity for phytate to form complexes, while Ca has the lowest affinity. Results obtained from the *in vitro* studies may, in part, explain the negative digestibilities of Zn and Cu in this study. The results of this study may indicate that the undigested phytate or phytic acid still bind a considerable amount of inorganic Zn and Cu. Supplementation of much more Mg, Cu, Fe, and Zn (via mineral premix) than the requirements of weanling pigs (Table II-2), and the unknown interactions among those dietary minerals (Adeola et al., 1995), may also explain our results of very low AFD of these selected minerals (Table II-6).

Phytase supplementation increased the AFD of ash. The increases were significant ($P < 0.05$) in Exp. 2, 3, and 4 (Table II-7). Obviously, the changes in the AFD of ash were associated with the changes in AFD of P and Ca. The AFD of DM, however, were not affected ($P > 0.05$) upon phytase supplementation. The AFD of DM depends not only on the AFD of ash but also on the AFD of organic matter. The changes in the AFD of ash in this study were obviously not large enough to affect the AFD of DM.

E. Implications

Supplementation of microbial phytase to the diets formulated with commonly used feed ingredients, even with inorganic P supplementation, can improve the utilization and decrease the fecal excretion of P in weanling pigs. The effect of phytase supplementation on the digestibility and retention of Ca varies with the composition of diets. No effect of phytase suplementation on the AFD of other minerals was detected. The rate of 500 FTU/kg diet is the recommended level of supplementation since the response to phytase supplementation was not further improved at 1,000 FTU/kg diet.

Table II-1. Formulation (%) of the basal diets[a]

Ingredients	Diets[b]			
	1	2	3	4
Corn	64.08	–	–	–
Wheat	–	68.51	55.72	–
Barley	–	–	–	30.10
Peas	–	–	–	39.50
Soybean meal	32.50	26.80	7.50	–
Canola meal	–	–	29.20	20.00
Canola oil	0.25	1.60	5.00	7.50
Limestone	0.97	1.25	0.74	0.55
Mono-dicalcium phosphate[c]	1.31	0.84	0.82	1.36
Salt	0.33	0.35	0.31	0.28
L-lysine-HCl	0.06	0.15	0.21	0.13
DL-methionine	–	–	–	0.03
L-threonine	–	–	–	0.05
L-tryptophan	–	–	–	0.013
Mineral premix[d]	0.10	0.10	0.10	0.10
Vitamin premix[d]	0.10	0.10	0.10	0.10
Chromic oxide	0.30	0.30	0.30	0.30

[a] As-fed basis.

[b] Diets 1, 2, 3 and 4 were used in Exp. 1, 2, 3 and 4, respectively. For each experiment, two more experimental diets were formulated by supplementation of phytase to the respective basal diet at rates of 500 and 1,000 FTU/kg.

[c] Supplied by Champion Feed Services Ltd, Westlock, AB, Canada. Contained P, 21.0%; Ca, 15.0%; F, 2.1 g/kg, and Fe, 9.0 g/kg.

[d] Supplied by Champion Feed Services Ltd, Westlock, AB, Canada. The mineral premix provided (per kilogram of diet): Ca, 57 mg; Fe, 150 mg; Zn, 150 mg; Mn, 40 mg; Cu, 25 mg; I, 0.5 mg; Co, 0.5 mg, and Se, 0.5 mg. The vitamin premix provided (per

kilogram of diet): vitamin A, 15,000 IU; vitamin D_3, 1,500 IU; vitamin E, 40 IU; vitamin K, 4 mg; thiamine, 2 mg; riboflavin, 6.5 mg; niacin, 40 mg; pyridoxine, 3 mg; pantothenic acid, 22 mg; folic acid, 2 mg; biotin, 0.1 mg, and vitamin B_{12}, 0.025 mg.

Table II-2. Chemical compositions of the basal diets[a]

Items	Diets[b]			
	1	2	3	4
Dry matter (%)	89.31	89.44	89.02	89.77
Gross energy (kcal/kg)	4,061	4,087	4,220	4,350
Metabolizable energy (kcal/kg)[c]	3,183	3,240	3,248	3,302
Crude protein (%)	20.45	21.31	20.33	18.93
Ash (%)	5.70	5.29	7.10	5.28
Minerals				
Phosphorus (total, %)	0.63	0.59	0.70	0.76
Phosphorus (available, %)[c]	0.36	0.35	0.35	0.36
Calcium (%)	0.94	0.84	1.05	0.70
Magnesium (g/kg)	1.67	1.78	2.63	1.88
Iron (mg/kg)	416.4	389.0	450.7	389.1
Manganese (mg/kg)	96.8	87.8	94.4	73.5
Copper (mg/kg)	31.2	25.4	29.3	25.1
Zinc (mg/kg)	217.8	195.1	194.2	185.4
Phytate-phosphorus (%)	0.199	0.199	0.215	0.158
Intrinsic phytase (FTU/kg)[d]	53	419	313	193
Phytase activities (FTU/kg)[e]	649	969	961	815
Phytase activities (FTU/kg)[f]	1130	1540	1440	924

[a] As-fed basis.

[b] Refer to Table II-1.

[c] Calculated, based on NRC (1998), without consideration of phytase effect.

[d] The intrinsic phytase activities in the basal diets.

[e] The total phytase activities after phytase supplementation at a rate of 500 FTU/kg diet.

[f] The total phytase activities after phytase supplementation at a rate of 1,000 FTU/kg diet.

Table II-3. Daily feed intakes and body weights of the pigs during the experiments[a]

Items	Initial[b]	Beginning of			Final[c]
		Period 1	Period 2	Period 3	
Daily feed intake (g/d)					
Exp. 1	–	337 ± 26.7	414 ± 27.1	493 ± 33.4	–
Exp. 2	–	369 ± 28.5	443 ± 33.9	552 ± 47.0	–
Exp. 3	–	341 ± 42.6	492 ± 39.7	618 ± 78.0	–
Exp. 4	–	394 ± 74.1	520 ± 97.3	699 ± 122.9	–
Body weights (kg)					
Exp. 1	6.6 ± 1.1	7.6 ± 0.8	10.1 ± 0.9	12.6 ± 1.1	15.1 ± 1.1
Exp. 2	7.6 ± 0.4	8.6 ± 0.9	11.0 ± 1.1	14.8 ± 1.7	17.7 ± 2.1
Exp. 3	5.6 ± 1.0	7.3 ± 1.1	9.8 ± 0.8	12.4 ± 1.6	16.3 ± 2.8
Exp. 4	7.6 ± 0.2	8.0 ± 1.5	10.5 ± 1.8	14.0 ± 2.5	16.8 ± 3.5

[a] Values are presented as mean ± SD.

[b] Initial body weights were measured at 3 wk of age. Prior to period 1, piglets were *ad libitum* fed a starter diet containing 20% CP.

[c] Final body weights were measured at the end of period 3.

Table II-4. Effect of phytase supplementation to different diets for weanling pigs on the apparent fecal digestibility, excretion and retention of phosphorus

Items	Supplementation rates (FTU/kg diet)			SEM[a]	*P*-value[b]
	0	500	1000		
Corn-soybean meal diet (Exp. 1)					
Intake (g/d)	2.62	2.66	2.59	0.027	
Digestibility (%)	48.9[c]	55.4[d]	58.4[d]	1.611	0.009
Fecal output (g/d)	1.34[c]	1.22[d]	1.09[e]	0.036	0.004
Urinary output (mg/d)	66.4	82.3	98.4	8.313	0.072
Retention (%)	46.4[c]	52.3[d]	54.5[d]	1.743	0.028
Wheat-soybean meal diet (Exp. 2)					
Intake (g/d)	2.69	2.71	2.66	0.030	
Digestibility (%)	56.8[c]	65.9[d]	69.5[e]	0.637	0.000
Fecal output (g/d)	1.16[c]	0.92[d]	0.81[e]	0.027	0.000
Urinary output (mg/d)	26.9	34.0	68.0	11.29	0.069
Retention (%)	55.9[c]	64.6[d]	67.0[d]	0.891	0.000
Wheat-soybean meal-canola meal diet (Exp. 3)					
Intake (g/d)	3.32	3.47	3.31	0.099	
Digestibility (%)	40.5[c]	49.0[d]	51.7[d]	1.698	0.004
Fecal output (g/d)	2.02[c]	1.80[cd]	1.60[d]	0.097	0.044
Urinary output (mg/d)	33.9	38.6	61.6	7.859	0.078
Retention (%)	39.5[c]	47.9[d]	49.6[d]	1.678	0.006
Barley-peas-canola meal diet (Exp. 4)					
Intake (g/d)	4.34	4.29	4.29	0.124	
Digestibility (%)	57.7[c]	69.6[d]	69.5[d]	1.556	0.001
Fecal output (g/d)	1.81[c]	1.33[d]	1.30[d]	0.056	0.000
Urinary output (mg/d)	162.9	165.9	177.3	23.49	0.902
Retention (%)	54.1[c]	65.6[e]	65.4[d]	1.820	0.003

[a] Standard error of the mean (n = 6).

[b] *P*-values obtained from the *F* test in ANOVA.

[c, d, e] Means in the same row with different superscripts differ ($P < 0.05$).

Table II-5. Effect of phytase supplementation to different diets for weanling pigs on the apparent fecal digestibility, excretion and retention of calcium

Items	Supplementation rates (FTU/kg diet) 0	500	1000	SEM[a]	*P*-value[b]
Corn-soybean meal diet (Exp. 1)					
Intake (g/d)	3.87	3.99	3.87	0.024	
Digestibility (%)	60.7	60.9	61.3	3.594	0.993
Fecal output (g/d)	1.55	1.68	1.61	0.126	0.774
Urinary output (mg/d)	90.9	89.8	88.4	14.223	0.992
Retention (%)	57.7	57.5	57.6	3.423	0.999
Wheat-soybean meal diet (Exp. 2)					
Intake (g/d)	3.81	3.83	3.76	0.042	
Digestibility (%)	71.3[c]	80.0[d]	80.1[d]	1.286	0.002
Fecal output (g/d)	1.11[c]	0.79[d]	0.76[d]	0.061	0.007
Urinary output (mg/d)	99.0	85.9	91.8	17.480	0.871
Retention (%)	68.6[c]	77.6[d]	77.7[d]	1.624	0.006
Wheat-soybean meal-canola meal diet (Exp. 3)					
Intake (g/d)	5.00	5.22	4.98	0.148	
Digestibility (%)	47.2	51.2	52.6	2.104	0.231
Fecal output (g/d)	2.69	2.60	2.35	0.147	0.297
Urinary output (mg/d)	79.6	81.0	61.2	8.086	0.217
Retention (%)	45.6	49.6	51.2	2.090	0.214
Barley-peas-canola meal diet (Exp. 4)					
Intake (g/d)	3.89	3.93	3.93	0.114	
Digestibility (%)	67.0[c]	75.9[d]	75.6[d]	2.382	0.049
Fecal output (g/d)	1.31	0.96	0.94	0.095	0.043
Urinary output (mg/d)	75.6	55.7	68.2	15.021	0.653
Retention (%)	65.1	74.3	73.5	2.352	0.045

[a] Standard error of the mean (n = 6).

[b] *P*-values obtained from the *F* test in ANOVA.

[c, d] Means in the same row with different superscripts differ ($P < 0.05$).

Table II-6. Effect of phytase supplementation to different diets for weanling pigs on the apparent fecal digestibilities (%) of magnesium, iron, manganese, copper and zinc

Items	Supplementation rates (FTU/kg diet) 0	500	1000	SEM[a]	*P*-value[b]
Corn-soybean meal diet (Exp. 1)					
Magnesium	36.4	36.5	37.2	2.442	0.965
Iron	7.8	-3.3	-3.5	5.889	0.349
Manganese	3.1	5.2	5.3	1.590	0.574
Copper	-17.3	-15.5	-14.2	3.364	0.819
Zinc	-53.7	-41.9	-48.7	6.649	0.490
Wheat-soybean meal diet (Exp. 2)					
Magnesium	45.6	49.3	53.7	1.475	0.015
Iron	8.6	7.6	4.7	2.004	0.407
Manganese	8.1	9.9	10.8	0.759	0.091
Copper	-16.4	-17.6	-16.2	1.870	0.846
Zinc	-19.7	-18.9	-12.2	3.666	0.331
Wheat-soybean meal-canola meal diet (Exp. 3)					
Magnesium	36.4	39.4	39.5	1.960	0.480
Iron	7.3	5.4	3.8	2.215	0.568
Manganese	3.4	4.1	4.0	1.073	0.895
Copper	-4.9	2.4	6.3	5.474	0.387
Zinc	-30.2	-29.5	-24.2	6.091	0.753
Barley-peas-canola meal diet (Exp. 4)					
Magnesium	29.5	34.2	29.0	1.510	0.074
Iron	6.7	5.9	4.2	1.985	0.673
Manganese	5.4	6.5	6.3	0.882	0.640
Copper	-12.5	-10.8	-11.8	1.907	0.830
Zinc	-19.6	-15.4	-18.8	2.816	0.562

[a] Standard error of the mean (n = 6).

[b] *P*-values obtained from the *F* test in ANOVA.

Table II-7. Effect of phytase supplementation to different diets for weanling pigs on the apparent fecal digestibilities (%) of ash and dry matter

Items	Supplementation level (FTU/kg diet)			SEM[a]	*P*-value[b]
	0	500	1000		
Corn-soybean meal diet (Exp. 1)					
Ash	57.6	58.7	60.1	1.623	0.569
Dry matter	88.2	88.4	88.4	0.210	0.799
Wheat-soybean meal diet (Exp. 2)					
Ash	61.8[c]	66.7[d]	68.2[d]	0.067	0.000
Dry matter	89.5	90.1	90.3	0.230	0.086
Wheat-soybean meal-canola meal diet (Exp. 3)					
Ash	51.2[c]	54.8[d]	55.2[d]	0.924	0.028
Dry matter	81.9	82.4	82.6	0.261	0.213
Barley-peas-canola meal diet (Exp. 4)					
Ash	52.9[c]	60.9[d]	60.3[d]	1.051	0.001
Dry matter	84.1	85.0	85.5	0.393	0.097

[a] Standard error of the mean (n = 6).

[b] *P*-values obtained from the *F* test in ANOVA.

[c, d] Means in the same row with different superscripts differ ($P < 0.05$).

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CHAPTER III. EFFECT OF PHYTASE SUPPLEMENTATION ON THE DIGESTIBILITIES OF CRUDE PROTEIN, AMINO ACIDS AND ENERGY IN DIFFERENT DIETS FED TO WEANLING PIGS [1]

A. Introduction

Abundant in many feed ingredients of plant origin, phytate/phytic acid is one of the most important anti-nutritional factors to nonruminant animals. The very limited ability of pigs to utilize phytate-phosphorus (P) poses at least three problems to swine producers, feed manufacturers, environmentalists, and the general public as well. The first problem involves the supplementation of inorganic P, which is expensive, to swine diets. Usually, inorganic P is over supplied to ensure that the animal requirement for bioavailable P is met. The second problem is environmental P pollution resulting from the excretion of a large proportion of P in swine manure. Growing concerns over this issue have been globally expressed, especially in Europe and North America. The third problem relates to the ability of phytate/phytic acid to form complexes with other dietary nutrients, such as minerals, proteins, free AA, and starch (Liao et al., 2002). Phytase is an enzyme that catalyzes the stepwise removal of orthophosphate groups from phytate/phytic acid by cleaving the chemical bonds within or between molecules of phytate/phytic acid (Maga, 1982), allowing the bound nutrients to be released for utilization. The activity of intrinsic phytase in diets for pigs and activity of endogenous phytase in the digestive tract are not sufficient for efficient hydrolysis of dietary phytate (Reddy et al., 1989; Cromwell et al., 1993).

Numerous investigations have shown that supplementation of microbial phytase to swine diets can improve the digestibility and retention of P in pigs (Simons et al., 1990;

[1] Portions of this study were presented at 2003 Banff Pork Seminar, Banff, AB, Canada, and the 9th International Symposium on Digestive Physiology in Pigs, 2003, Banff, AB, Canada.

Cromwell et al., 1993; Lei et al., 1993a, b). The recommended supplementation rate appears to be 500 phytase units (**FTU**) per kilogram of diet, although a maximum response can be achieved at 1,000 FTU/kg diet (Khan, 1995; Jongbloed et al., 2000). Some studies have shown that the supplementation of phytase to diets for pigs also improved the apparent ileal digestibilities (**AID**) of CP and individual AA (e.g., Mroz et al., 1994; Kemme et al., 1999). Other studies have shown no effect of phytase supplementation on the AID of CP and AA (e.g., Valaja et al., 1998; Sands, 2002). The lack of consistency in response to phytase supplementation was recently discussed by Adeola and Sands (2003) and they caution in the use of any overly simplistic guidelines that ascribe an "AA response factor" to phytase supplementation.

The objective of this study was to determine the effect of microbial phytase supplementation, at rates of 500 and 1,000 FTU/kg, on the AID of CP and AA and the apparent fecal digestibilities (**AFD**) of CP and energy to diets for weanling pigs. The diets were formulated to contain commonly used feed ingredients.

B. Experimental Procedures

Experiments and Diets

Four experiments were carried out with weanling pigs. In each experiment, the piglets were fed a basal diet consisting of ingredients commonly used in western Canada (Table III-1). To each basal diet, *Aspergillus niger* phytase (Natuphos®, DSM Food Specialties, Delft, The Netherlands) was supplemented at rates of 500 and 1,000 FTU/kg to formulate two more experimental diets. One FTU is defined as the quantity of enzyme that liberates 1 mmol of ortho-phosphate per minute from 5.1 mM Na-phytate at pH 5.5 and 37°C (Engelen et al., 2001). The diets were supplemented with inorganic P (a mixture of mono- and dicalcium phosphate) to meet the NRC (1998) standards for available P, which is 0.32% for weanling pigs. Canola oil was included in the diets to reduce the dustiness and to increase the digestible energy content up to the level recommended by NRC (1998). The calculated metabolizable energy (ME) contents, based on NRC (1998), of the basal diets for Exp. 1, 2, 3 and 4 were 3,183, 3,240, 3,248

and 3,302 kcal/kg, respectively. Free AA were supplemented, when necessary, to meet the NRC (1998) standards on the basis of their apparent ileal digestible supply. Vitamins and minerals were supplemented to meet or exceed the NRC (1998) standards. Chromic oxide was included in the diet at a rate of 0.30% as the digestibility indicator.

As shown in Table III-1, the major ingredients of the basal diet for Exp. 1 were corn and soybean meal. For Exp. 2, the major ingredients were wheat and soybean meal. For Exp. 3, the major ingredients were wheat, soybean meal and canola meal. For Exp. 4, the major ingredients were barley, peas and canola meal. The major ingredients were ground through a 2-mm mesh sieve prior to diet formulation. Prior to surgery and during the recuperation period, the piglets were fed *ad libitum* a starter diet containing 20% CP. All diets were fed in the form of mash. Water was freely available from a low-pressure drinking nipple.

Animal Trial Procedures

For each of the four experiments, six PIC barrows (Camborough × Canabrid), weaned at three weeks of age, were obtained from the University of Alberta Swine Research Unit. The barrows were housed individually in metabolism crates (height: 82 cm; length: 124 cm; width: 76 cm) in a barn, in which the temperature was maintained between 25 and 28°C. On d 6 and 7 after weaning and adjustment to the crates, each piglet was fitted with a simple T-cannula at the distal ileum, approximately 5 cm from the ileo-cecal sphincter. Detailed descriptions of cannula preparation, surgery, pre- and post-operative care were previously given by Sauer (1976) and Li et al. (1993).

Following a 1-wk recuperation period after surgery, the piglets were fed three experimental diets according to a repeated 3 × 3 Latin square design. Each experimental period comprised 14 d. For Exp. 1 and 2, the diets were fed to the piglets at a rate of 2.4 times the maintenance requirement for ME (i.e., 100 kcal/kg of $BW^{0.75}$), based on the individual BW of each piglet which was determined at the beginning of each experimental period (Table III-3). For Exp. 3 and 4, the diets were fed to the piglets at a rate of 5% of BW (equivalent to 2.7, 2.9, and 3.1 times the maintenance requirement for ME in periods 1, 2, and 3 respectively). The daily meal allowances were offered twice daily at 0800 and 2000, equal amounts each meal.

The animals used in this study were cared for in accordance with the guidelines established by the CCAC (1993), and the experimental proposal was approved by the Faculty of Agriculture, Forestry and Home Economics Animal Care Committee of the University of Alberta.

Sample Collection and Chemical Analysis

Samples of the major feed ingredients were taken prior to diet formulation. Samples of the diets were taken during the time the meal allowances were prepared. The collection of feces was initiated at 0800 on d 8 of each experimental period and continued for 96 consecutive hours. Feces were collected by aid of colostomy bags (Stomahesive® Wafer, Sur-Fit® Natura™, ConvaTec, Princeton, NJ, USA). The bags were changed every 4 to 8 h depending on the amount of feces collected. Feces were frozen at –28°C immediately after collection. Ileal digesta were collected into a soft plastic tube (length, 15 cm; i.d., 4 cm) for 36 h; from 0800 to 2000 on d 12, 13, and 14, respectively. Prior to collection, 5 mL 10% (vol/vol) formic acid solution was placed into each tube. The tube was attached to the barrel of the cannula with a rubber band, and was removed and replaced as soon as it was nearly filled with digesta. Digesta were frozen at –28°C immediately after collection. Detailed procedures for collection of ileal digesta were previously described by Sauer (1976) and Li et al. (1993).

Prior to chemical analysis, feces and digesta were pooled leaving one sample for each pig in each experimental period. Feces were air-dried, and digesta were freeze-dried. The dried samples of feces and digesta, and samples of ingredients and diets were ground through a 0.5-mm mesh screen in a Thomas-Wiley Laboratory Mill (Arther H. Thomas Co., Philadelphia, PA, USA).

Crude protein (N × 6.25) was measured with a Leco FP-428 Nitrogen Determinator (Leco® Corporation, St. Joseph, MI, USA). Gross energy was determined with an AC-300 Leco Automatic Calorimeter. The phytate P contents in the basal diets were analyzed according to the procedures described by Haug and Lantzsch (1983). The intrinsic phytase activities (FTU/kg diet) were analyzed with a colorimetric enzymatic procedure according to AOAC International official method 2000.12 (Engelen et al., 1994, 2001). Chromic oxide was determined with a spectrophotometric procedure

according to Fenton and Fenton (1979). Analyses of ingredients and diets were carried out in triplicate; analyses of feces and digesta in duplicate.

For AA analyses, approximately 0.05 g of finely ground digesta (< 0.1 mm) was weighed into a screw-capped culture tube, mixed with 3 mL of 6 N HCl, and hydrolyzed at 110°C for 24 h in an oven. The hydrolyzed samples were mixed with an internal standard, DL-amino-n-butyric acid, and centrifuged at 1,110 × g for 15 min at 4°C. The supernatant of the sample was analyzed using a Varian 5000 high performance liquid chromatography system with a reverse-phase column and a Varian Fluorichrom detector (Varian Canada Inc., Mississauga, ON) according to principles outlined by Jones and Gilligan (1983). The AA were derivatized with an *o*-phthaldialdehyde reagent solution. The mobile phase consisted of two solvents (A and B) with a flow rate of 1.1 ml/min. Solvent A was a 0.1 M sodium acetate (pH 7.2) contained methanol and tetrahydrofuran in a ratio of 90 to 5. Solvent B was pure methanol. Peaks were recorded and integrated using the Ezchrom™ Chromatography Data System (version 4.2; Shimadzu Scientific Instruments Inc., Columbia, MD, USA). The procedure was described in detail by Sedgwick et al. (1991). Cysteine, proline, and tryptophan were not determined.

Digestibility Calculations and Statistical Analysis

The AID of CP and AA, and the AFD of CP and energy were calculated by using the following equation:

$$D_D = 100\% - [(A_F \times I_D) / (A_D \times I_F)] \times 100\%$$

where D_D is the apparent digestibility of a nutrient or energy in the assay diet (%); A_F is the nutrient or energy concentration in ileal digesta or feces (%); I_D is the indicator concentration in the assay diet (%); A_D is the nutrient or energy concentration in the assay diet (%); I_F is the indicator concentration in ileal digesta or feces (%).

Based on the following linear model, the digestibility data were subjected to statistical analysis using the General Linear Model Procedure of SAS® (1990).

$$Y_{ijk} = \mu + T_i + P_j + A_k + \varepsilon_{ijk}$$

where Y_{ijk} is a digestibility value; μ is the overall mean of the digestibility values; T_i is the fixed effect of treatments (i.e. phytase supplementation) and i = 1, 2, 3; P_j is the random effect of experiment periods and j = 1, 2, 3; A_k is the random effect of animals

and $k = 1, 2, 3, 4, 5, 6$; ε_{ijk} is the residual experimental error with $N(0, \sigma^2)$. After analysis of variance, means of treatments were compared using the Student-Newman-Keuls multiple range test. Probability levels of $P \leq 0.05$ and $0.05 < P \leq 0.10$ were defined as significant differences and tendencies, respectively.

C. Results

Diet Analysis and Animal Health

The chemical compositions of the four basal diets are presented in Table III-2. The analyzed values of the nutrients in the diets were close to the values calculated based on the analyzed values in the ingredients. The phytate-P contents of the basal diets ranged between 0.16 and 0.22% (as-fed basis), while the intrinsic phytase activities ranged between 53 and 419 FTU/kg diet. The content of CP in diet 4 was 18.9% and lower than in the other diets which ranged from 20.3 to 21.3%. Diet 4 included free methionine, threonine, and tryptophan (Table III-1).

All piglets remained healthy and usually consumed their meal allowances within 30 min after feeding throughout the experiments. The average BW at the initiation of the experiments, at the beginning of each experimental period, and at the conclusion of the experiments are presented in Table III-2. The ADG of pigs in Exp. 1, 2, 3 and 4 were 178, 221, 216 and 211 g/d, respectively. Postmortem examinations, conducted at the conclusion of each experiment, revealed no adhesions or any other intestinal abnormalities.

Apparent Ileal Digestibilities of Crude Protein and Amino Acids

Experiment 1. The effect of phytase supplementation to the corn-soybean meal diet on the AID of CP and AA is presented in Table III-4. There were no differences ($P > 0.10$) in the AID of CP and AA between the experimental diets. However, numerical increases were found upon phytase supplementation for the AID of CP and nearly all AA. Of the indispensable AA, the increases upon phytase supplementation ranged from 0.2

(threonine) to 1.4 (phenylalanine) percentage units (**pu**). The AID of CP was increased by 0.2 to 0.8 pu.

Experiment 2. The effect of phytase supplementation to the wheat-soybean meal diet on the AID of CP and AA is presented in Table III-5. There were no differences ($P > 0.10$) in the AID of CP and AA between the experimental diets. However, numerical increases were found for the AID of CP and nearly all AA upon phytase supplementation. Of the indispensable AA, the increases upon phytase supplementation at the rate of 1,000 FTU/kg diet ranged from 0.1 (threonine) to 1.2 (lysine) pu. The AID of CP was increased by 0.3 to 1.2 pu.

Experiment 3. The effect of phytase supplementation to the wheat-soybean meal-canola meal diet on the AID of CP and AA is presented in Table III-6. No differences ($P > 0.05$) were detected in the AID of CP and AA between the experimental diets. As in the previous experiments, there were numerical increases ($P > 0.06$) in the AID of CP and AA upon phytase supplementation. The increases in the AID of histidine and glycine approached significance ($P < 0.10$). Of the indispensable AA, the increases upon phytase supplementation ranged from 2.3 (arginine) to 3.9 (phenylalanine) pu. Of the dispensable AA, the increases ranged from 1.8 (glutamic acid) to 4.3 (glycine) pu. The AID of CP was increased by 2.7 to 2.8 pu.

Experiment 4. The effect of phytase supplementation to the barley-peas-canola meal diet on the AID of CP and AA is presented in Table III-7. No differences ($P > 0.10$) were detected in the AID of CP and AA between the experimental diets. As in the other experiments, there were numerical increases in the AID of CP and most AA. Of the indispensable AA, the increases ranged from 0.1 (arginine) to 1.4 (lysine) pu. The AID of CP was increased by 0.2 to 0.6 pu.

Apparent Fecal Digestibilities of Crude Protein and Energy

The AFD of CP and the contents of digestible energy (**DE**) for all the diets are presented in Table III-8. The DE contents were calculated from the AFD of energy. As was expected, the AFD of CP were higher than their corresponding AID for all the diets, ranging from 6.7 to 12.0 pu with an average ± SD of 8.7 ± 1.9 pu (Table III-8 vs. Tables III-4, III-5, III-6 and III-7). It needs to be pointed out here that there are no differences

between the AFD of nutrients and energy obtained from cannulated and non-cannulated pigs (Sauer and Ozimek, 1986).

Phytase supplementation at either rate to either diet did not affect ($P > 0.10$) the AFD of CP and of energy in all diets for all experiments, although some small numerical increases ($P = 0.11$ to 0.54) were found upon phytase supplementation (Table III-8).

Nitrogen Balance Study

As shown in Table III-9, there was no significant effect ($P > 0.10$) of phytase supplementation on the fecal output, urinary output, and retention of nitrogen in all the four diets tested in four experiments.

D. Discussion

Many studies have shown that the supplementation of microbial phytase will improve the utilization of phytate P in feedstuffs of plant origin for pigs. However, as was pointed out by Adeola and Sands (2003), supplementation of diets with microbial phytase does not consistently improve the digestibilities of nutrients other than P that may be bound to phytate/phytic acid. The other nutrients include protein/AA. They caution for the use of any oversimplistic guidelines that ascribe an "AA response factor" to phytase supplementation. On a theoretical basis, four mechanisms can be suggested for a possible negative effect of dietary phytate/phytic acid on the AID of protein and AA: 1) inherent phytate-protein/AA complexes in feedstuffs, 2) *de novo* formation of phytate-protein complexes in the digestive tract, 3) *de novo* formation of phytate-AA complexes after digestion of dietary protein in the digestive tract, and 4) formation of complexes between phytate and proteolytic enzymes in the digestive tract (Kies et al., 1997; Selle et al., 2000). Conceptually, the extent to which phytase supplementation will increase the AID of CP/AA will be dependent, in part, on the proportion of protein/AA bound in phytate bonds. But, as was discussed by Kemme et al. (1999), there are many other factors that should be considered, namely the type of protein, the solubility of protein, *in situ* pH, the contents of other dietary minerals, and three-way interactions between phytate, proteolytic enzymes and protein/AA in the digestive contents.

There were no significant improvements ($P > 0.05$) in the AID of CP and AA upon phytase supplementation, at both rates of 500 and 1,000 FTU/kg, to the four diets evaluated in this study (Tables III-4, III-5, III-6, and III-7). The largest numerical improvements were found in Exp. 3 when phytase was supplemented to a wheat-canola meal-soybean meal diet (Table III-6).

The effect of phytase supplementation on the AID of AA was also examined in other studies with growing pigs (Officer and Batterham, 1992a, b; Mroz et al., 1994; Kornegay et al., 1998; Valaja et al., 1998; Kemme et al., 1999; Traylor et al., 2001; Rice, 2002; Sands, 2002). Phytase supplementation to a "wet barley protein with fiber" diet (250 and 500 FTU/kg) did not affect ($P > 0.10$) the AID of AA in the study by Valaja et al. (1998). The study by Rice (2002) also showed no effect ($P > 0.10$) of phytase supplementation to an 11% CP diet on the AID of AA. Further, Sands (2002) reported no differences ($P > 0.10$) in the AID of AA when phytase (1,200 FTU/kg) was supplemented to either a low (0.22%) or a high (0.39%) phytate-P diet. As well, Traylor et al. (2001) reported no increases ($P > 0.05$) in the AID of AA when phytase (500 to 1,500 FTU/kg) was supplemented to a semi-purified soybean meal diet.

Mroz et al. (1994) and Kemme et al. (1999) reported small increases in the AID of AA upon phytase supplementation. Of the indispensable AA, these increases were significant ($P < 0.05$) for arginine (2.5 pu) and methionine (3.9 pu) in the study by Mroz et al. (1994), and for isoleucine (2.1 pu), lysine (2.4 pu), threonine (2.9 pu) and tryptophan (4.4 pu) in the study by Kemme et al. (1999). In the study by Mroz et al. (1994), phytase was supplemented (800 FTU/kg) to a corn-tapioca-soybean meal-barley-peas diet. In the study by Kemme et al. (1999) phytase was supplemented (900 FTU/kg) to a corn-soybean meal diet. Kornegay et al. (1998) reported small linear increases in the AID of the indispensable AA when phytase, at rates of 250 FTU/kg and 500 FTU/kg, was supplemented to a corn-soybean meal diet. The linear increases were significant ($P < 0.05$) for valine, isoleucine, and arginine. Supplementation of phytase at a rate of 500 FTU/kg diet increased the AID of the indispensable AA from 1.9 to 4.0 pu. On the other hand, Officer and Batterham (1992a) reported relatively large increases in the AID of the indispensable plus semi-indispensable AA when phytase was supplemented to a semi-purified diet containing 40% Linola™ meal for growing pigs. The AID of the AA

increased by 4.0 to 13.0 pu. However, only the increases for lysine (12 pu) and histidine (12 pu) were significant ($P < 0.05$).

Both the results from the published studies in which the effect of phytase supplementation on the AID of AA were determined, and the results of this study are summarized in Table III-10. For simplicity, the average of the AID of the indispensable AA of the control and the phytase-supplemented diets are presented, in addition to the differences. In studies in which phytase was supplemented at more than one rate, the highest averages of the AID of the indispensable AA are indicated. Perhaps it may have been more appropriate to present the AID of the limiting AA rather than the average of the AID of the indispensable AA, as the AID of the limiting AA usually determine the utilization of dietary protein. However, this was not done for reasons that in some studies the requirements of AA were met. It should also be pointed out that the AID of tryptophan was not determined in some of the studies. Further, in some studies analyses of methionine were not carried out according to the correct procedure. With the exception of the study by Officer and Batterham (1992a), the increases upon phytase supplementation in the average AID of the indispensable AA were of small magnitudes. Officer and Batterham (1992a) suggested that a substantial proportion of protein/AA in Linola™ meal may be bound by phytate bonds. However, some care should be execised in the interpretation of their results. First of all, the slaughter method was used to determine the AID of AA. This method for determining the AID of AA is open to criticism. Secondly, the publication by Officer and Batterham (1992a) and a related one (Officer and Batterham, 1992b) only provided very limited information on the experimental procedures that were employed.

Not taking into account results reported by Officer and Batterham (1992a, b), the results from this study and those from the literature show that the supplementation of phytase usually increases the AID of AA. As shown in Table III-10, illustrating the averages of the AID of the indispensable AA, these increases are of a very small magnitude (0.5 to 2.9 pu). As was reviewed previously, these small increases reached significance ($P < 0.05$) for some of the indispensable AA in some studies.

As was suggested by Liao et al. (2002), in theory the largest response to phytase suplementation is to be expected when the diet is high in phytate/phytic acid and low in

intrinsic phytase activity. As was discussed previously, Sands (2002) reported no differences ($P > 0.10$) in the AID of AA when phytase was supplemented to a low- or a high-phytate-P diet. These results were confirmed by Liao et al. (2004) in a recent study, in which phytase was supplemented to a high- and a low-phytate-P diets fed to growing pigs. The high-phytate diet contained 20% rice bran which is rich in phytate-P. Suplementation of phytase (1,000 FTU/kg) to both diets did not affect ($P > 0.10$) the AID of CP and AA. Consistent with most reports in the literature there were small increases in the AID of CP and AA. The study by Liao et al. (2004) showed that a response to phytase supplementation on the AID of CP and AA is independent of the dietary content of phytate-P.

In conclusion, this present study showed that there was no significant effect ($P > 0.05$) of phytase supplementation, at rates of 500 and 1,000 FTU/kg, on the AID of AA in different diets for weanling pigs. Consistent with most reports in the literature, there were small but non-significant increases in the AID of CP and AA.

E. Implications

Supplementation of microbial phytase to the diets formulated with commonly used feed ingredients in Canada for weanling pigs does not improve the AID of CP and AA significantly ($P > 0.05$). Consistent with many other reports in the literature, there were small increases ($P > 0.05$) in the AID of CP and AA. Also, there were very small improvements ($P > 0.10$) in the AFD of CP and energy.

Table III-1. Formulation (%) of the four basal diets[a]

Ingredients	Diets[b]			
	1	2	3	4
Corn	64.08	–	–	–
Wheat	–	68.51	55.72	–
Barley	–	–	–	30.10
Peas	–	–	–	39.50
Soybean meal	32.50	26.80	7.50	–
Canola meal	–	–	29.20	20.00
Canola oil	0.25	1.60	5.00	7.50
Limestone	0.97	1.25	0.74	0.55
Mono-dicalcium phosphate[c]	1.31	0.84	0.82	1.36
Salt	0.33	0.35	0.31	0.28
Lysine-HCl	0.06	0.15	0.21	0.13
DL-methionine	–	–	–	0.03
L-threonine	–	–	–	0.05
L-tryptophan	–	–	–	0.013
Mineral premix[d]	0.10	0.10	0.10	0.10
Vitamin premix[d]	0.10	0.10	0.10	0.10
Chromic oxide	0.30	0.30	0.30	0.30

[a] As-fed basis.

[b] Diets 1, 2, 3 and 4 were used in Exp. 1, 2, 3 and 4, respectively. For each experiment, two more experimental diets were formulated by supplementation of phytase to the respective basal diet at rates of 500 and 1,000 FTU/kg.

[c] Supplied by Champion Feed Services Ltd, Westlock, AB, Canada. Contained P, 21.0%; Ca, 15.0%; F, 2.1 g/kg, and Fe, 9.0 g/kg.

[d] Supplied by Champion Feed Services Ltd, Westlock, AB, Canada. The mineral premix provided (per kilogram of diet): Ca, 57 mg; Fe, 150 mg; Zn, 150 mg; Mn, 40 mg; Cu, 25 mg; I, 0.5 mg; Co, 0.5 mg, and Se, 0.5 mg. The vitamin premix provided (per

kilogram of diet): vitamin A, 15,000 IU; vitamin D_3, 1,500 IU; vitamin E, 40 IU; vitamin K, 4 mg; thiamine, 2 mg; riboflavin, 6.5 mg; niacin, 40 mg; pyridoxine, 3 mg; pantothenic acid, 22 mg; folic acid, 2 mg; biotin, 0.1 mg; and vitamin B_{12}, 0.025 mg.

Table III-2. Chemical compositions of the four basal diets[a]

Items	Diets[b]			
	1	2	3	4
Dry matter (%)	89.31	89.44	89.02	89.77
Gross energy (kcal/kg)	4,061	4,087	4,220	4,350
Metabolizable energy (kcal/kg)[c]	3,183	3,240	3,248	3,302
Crude protein (%)	20.45	21.31	20.33	18.93
Phosphorus (total, %)	0.63	0.59	0.70	0.76
Phosphorus (available, %)[c]	0.36	0.35	0.35	0.36
Calcium (%)	0.94	0.84	1.05	0.70
Indispensable amino acids (%)				
Arginine	1.27	1.19	1.03	1.31
Histidine	0.52	0.47	0.46	0.48
Isoleucine	0.97	0.99	0.89	0.86
Leucine	1.80	1.59	1.46	1.54
Lysine	1.09	1.12	1.13	1.30
Methionine	0.29	0.27	0.34	0.28
Phenylalanine	1.10	1.05	0.88	0.96
Threonine	0.76	0.68	0.70	0.71
Valine	1.01	1.05	1.07	1.03
Dispensable amino acids (%)				
Alanine	1.05	0.87	0.84	0.90
Aspartic acid	1.96	1.87	1.52	1.61
Glutamic acid	3.81	5.06	4.38	3.64
Glycine	0.98	0.92	0.88	1.18
Serine	0.74	0.80	0.71	0.75
Tyrosine	0.51	0.48	0.57	0.58
Phytate-phosphorus (%)	0.20	0.20	0.22	0.16
Intrinsic phytase (FTU/kg)[d]	53	419	313	193

Phytase activities (FTU/kg)[e]	649	969	961	815
Phytase activities (FTU/kg)[f]	1130	1540	1440	924

[a] As-fed basis.

[b] Refer to Table III-1.

[c] Calculated, based on NRC (1998), without consideration of phytase effect.

[d] The intrinsic phytase activities in the basal diets.

[e] The total phytase activities after supplementation of phytase at a rate of 500 FTU/kg.

[f] The total phytase activities after supplementation of phytase at a rate of 1,000 FTU/kg.

Table III-3. Daily feed intakes and body weights of the pigs during the experiments[a]

Items	Initial[b]	Beginning of Period 1	Beginning of Period 2	Beginning of Period 3	Final[c]
Daily feed intake (g/d)					
Exp. 1	–	337 ± 26.7	414 ± 27.1	493 ± 33.4	–
Exp. 2	–	369 ± 28.5	443 ± 33.9	552 ± 47.0	–
Exp. 3	–	341 ± 42.6	492 ± 39.7	618 ± 78.0	–
Exp. 4	–	394 ± 74.1	520 ± 97.3	699 ± 122.9	–
Body weights (kg)					
Exp. 1	6.6 ± 1.1	7.6 ± 0.8	10.1 ± 0.9	12.6 ± 1.1	15.1 ± 1.1
Exp. 2	7.6 ± 0.4	8.6 ± 0.9	11.0 ± 1.1	14.8 ± 1.7	17.7 ± 2.1
Exp. 3	5.6 ± 1.0	7.3 ± 1.1	9.8 ± 0.8	12.4 ± 1.6	16.3 ± 2.8
Exp. 4	7.6 ± 0.2	8.0 ± 1.5	10.5 ± 1.8	14.0 ± 2.5	16.8 ± 3.5

[a] Values are presented as mean ± SD.

[b] Initial body weights were determined at 3 wk of age. Prior to period 1, piglets were fed *ad libitum* a starter diet containing 20% CP.

[c] Final body weights were measured at the end of period 3.

Table III-4. Effect of phytase supplementation on the apparent ileal digestibilities (%) of crude protein and amino acids in the corn-soybean meal diet (Exp. 1)

Items	Supplementation level (FTU/kg diet)			SEM[a]	*P*-value[b]
	0	500	1000		
Crude protein	76.0	76.2	76.8	1.38	0.910
Indispensable amino acids					
Arginine	86.4	86.8	86.6	0.86	0.949
Histidine	83.6	83.2	83.5	1.31	0.977
Isoleucine	79.3	80.5	80.6	1.34	0.750
Leucine	78.0	76.1	78.8	2.22	0.704
Lysine	75.8	75.5	76.4	1.68	0.934
Methionine	88.5	89.4	89.4	0.84	0.661
Phenylalanine	82.1	83.1	83.5	1.22	0.728
Threonine	73.1	73.1	73.3	1.12	0.995
Valine	75.7	76.5	76.7	1.56	0.881
Dispensable amino acids					
Alanine	76.4	77.1	77.5	1.57	0.875
Aspartic acid	76.8	77.6	77.5	1.46	0.905
Glutamic acid	83.6	84.0	84.2	1.27	0.934
Glycine	73.1	72.5	73.0	1.12	0.929
Serine	77.0	77.4	77.7	1.31	0.925
Tyrosine	82.0	82.9	83.6	1.27	0.707

[a] Standard error of the mean (n = 6).

[b] *P*-values obtained from the *F* test in ANOVA.

Table III-5. Effect of phytase supplementation on the apparent ileal digestibilities (%) of crude protein and amino acids in the wheat-soybean meal diet (Exp. 2)

Items	Supplementation level (FTU/kg diet)			SEM[a]	*P*-value[b]
	0	500	1000		
Crude protein	83.3	83.6	84.5	0.65	0.433
Indispensable amino acids					
Arginine	88.8	89.3	89.5	0.49	0.634
Histidine	86.0	85.8	86.0	0.69	0.969
Isoleucine	85.8	85.6	86.4	0.60	0.662
Leucine	85.7	85.7	86.4	0.62	0.715
Lysine	84.8	84.9	86.0	0.78	0.531
Methionine	89.7	89.3	90.3	0.63	0.579
Phenylalanine	86.7	86.6	87.3	0.56	0.645
Threonine	78.2	77.5	78.3	1.08	0.860
Valine	83.5	83.2	83.9	0.69	0.768
Dispensable amino acids					
Alanine	79.5	79.2	80.3	0.96	0.719
Aspartic acid	81.8	81.7	82.7	0.86	0.681
Glutamic acid	91.2	91.2	92.3	0.46	0.225
Glycine	76.0	76.2	76.6	1.55	0.960
Serine	84.2	83.6	84.4	0.63	0.687
Tyrosine	86.2	86.2	87.1	0.62	0.532

[a] Standard error of the mean (n = 6).

[b] *P*-values obtained from the *F* test in ANOVA.

Table III-6. Effect of phytase supplementation on the apparent ileal digestibilities (%) of crude protein and amino acids in the wheat-soybean meal-canola meal diet (Exp. 3)

Items	Supplementation level (FTU/kg diet)			SEM[a]	*P*-value[b]
	0	500	1000		
Crude protein	72.0	74.8	74.7	1.10	0.197
Indispensable amino acids					
Arginine	81.9	84.5	84.2	0.86	0.122
Histidine	81.8	85.2	85.5	1.01	0.064
Isoleucine	75.4	78.8	78.3	1.25	0.176
Leucine	77.9	81.0	80.3	1.11	0.182
Lysine	77.0	80.4	79.8	1.09	0.131
Methionine	84.7	87.5	87.6	1.12	0.190
Phenylalanine	78.2	82.1	81.1	1.25	0.128
Threonine	69.0	72.4	72.4	1.20	0.131
Valine	73.4	77.2	76.4	1.27	0.146
Dispensable amino acids					
Alanine	72.5	75.9	75.4	1.32	0.209
Aspartic acid	71.1	73.7	73.5	1.12	0.240
Glutamic acid	86.3	88.4	88.1	0.64	0.105
Glycine	68.1	72.4	71.5	1.22	0.087
Serine	72.6	76.2	75.6	1.22	0.142
Tyrosine	77.5	79.7	79.7	0.94	0.208

[a] Standard error of the mean (n = 6).

[b] *P*-values obtained from the *F* test in ANOVA.

Table III-7. Effect of phytase supplementation on the apparent ileal digestibilities (%) of crude protein and amino acids in the barley-peas-canola meal diet (Exp. 4)

Items	Supplementation level (FTU/kg diet)			SEM[a]	*P*-value[b]
	0	500	1000		
Crude protein	73.3	73.5	73.9	1.46	0.954
Indispensable amino acids					
Arginine	85.4	85.2	85.5	0.99	0.980
Histidine	83.5	82.4	83.1	1.28	0.843
Isoleucine	76.5	76.6	77.5	1.20	0.822
Leucine	79.1	79.0	79.6	1.32	0.940
Lysine	81.8	82.0	83.2	1.47	0.749
Methionine	83.5	83.2	83.1	1.06	0.948
Phenylalanine	80.0	79.9	80.7	1.18	0.873
Threonine	67.7	68.1	68.8	1.57	0.878
Valine	74.1	74.1	74.8	1.26	0.893
Dispensable amino acids					
Alanine	72.0	73.0	72.0	2.01	0.914
Aspartic acid	74.2	74.6	75.1	1.21	0.876
Glutamic acid	85.2	85.4	85.4	0.88	0.985
Glycine	62.2	61.5	64.3	2.79	0.776
Serine	72.1	71.9	72.6	1.78	0.969
Tyrosine	76.6	77.2	77.3	1.64	0.953

[a] Standard error of the mean (n = 6).

[b] *P*-values obtained from the *F* test in ANOVA.

Table III-8. Effect of phytase supplementation on the apparent fecal digestibilities (%) of crude protein and the digestible energy (DE) contents of the experimental diets[a]

Items	Supplementation level (FTU/kg diet)			SEM[b]	*P*-value[c]
	0	500	1000		
Corn-soybean meal diet (Exp. 1)					
Crude protein	87.1	88.2	87.3	0.46	0.278
DE (kcal/kg)	3,587	3,605	3,596	5.74	0.157
Wheat-soybean meal diet (Exp. 2)					
Crude protein	90.3	91.1	91.3	0.55	0.437
DE (kcal/kg)	3,653	3,672	3,677	14.4	0.473
Wheat-soybean meal-canola meal diet (Exp. 3)					
Crude protein	82.5	83.4	83.6	0.33	0.111
DE (kcal/kg)	3,494	3,511	3,507	10.3	0.535
Barley-peas-canola meal diet (Exp. 4)					
Crude protein	80.3	80.2	81.6	0.92	0.501
DE (kcal/kg)	3,633	3,664	3,679	21.7	0.254

[a] The DE contents were calculated from the AFD of energy.

[b] Standard error of the mean (n = 6).

[c] *P*-values obtained from the *F* test in ANOVA.

Table III-9. Effect of phytase supplementation to different diets for weanling pigs on nitrogen balance

Items	Supplementation rates (FTU/kg diet) 0	500	1000	SEM[a]	*P*-value[b]
Corn-soybean meal diet (Exp. 1)					
Intake (g/d)	13.4	13.8	13.4	0.08	
Digestibility (%)	87.1	88.2	87.3	0.46	0.278
Fecal output (g/d)	1.72	1.64	1.68	0.05	0.526
Urinary output (g/d)	2.09	1.99	1.90	0.23	0.856
Retention (%)	71.5	73.8	72.9	1.51	0.586
Wheat-soybean meal diet (Exp. 2)					
Intake (g/d)	15.6	15.6	15.3	0.17	
Digestibility (%)	90.3	91.1	91.3	0.55	0.437
Fecal output (g/d)	1.47	1.34	1.32	0.09	0.417
Urinary output (g/d)	2.78	2.88	2.72	0.12	0.655
Retention (%)	72.6	72.8	73.4	0.86	0.796
Wheat-soybean meal-canola meal diet (Exp. 3)					
Intake (g/d)	14.7	15.4	15.3	0.49	
Digestibility (%)	82.5	83.4	83.6	0.33	0.111
Fecal output (g/d)	2.55	2.52	2.50	0.10	0.941
Urinary output (g/d)	1.64	1.90	1.86	0.22	0.688
Retention (%)	71.3	71.0	70.9	1.25	0.980
Barley-peas-canola meal diet (Exp. 4)					
Intake (g/d)	16.8	16.5	17.1	0.38	
Digestibility (%)	80.3	80.2	81.6	0.92	0.501
Fecal output (g/d)	3.36	3.21	3.12	0.14	0.511
Urinary output (g/d)	2.14	1.31	1.71	0.43	0.438
Retention (%)	68.3	72.0	71.0	2.53	0.586

[a] Standard error of the mean (n = 6).

[b] *P*-values obtained from the *F* test in ANOVA.

Table III-10. Effect of phytase supplementation on the average of the apparent ileal digestibilities (%) of the indispensable amino acids calculated from the results reported in the literature and this study

References	Apparent ileal digestibilities		
	Control diet	Phytase diet	Differences (n)[a]
Officer and Batterham (1992a)	62.0	70.6	8.6 ± 3.0 (8)
Mroz et al. (1994)	78.7	79.4	0.7 ± 1.7 (10)
Kornegay et al. (1998)	75.6	78.2	2.6 ± 0.9 (9)
Valaja et al. (1998)	83.0	81.6	-1.4 ± 0.8 (9)
Kemme et al. (1999)	77.1	79.3	2.2 ± 1.0 (10)
Traylor et al. (2001)	87.5	88.3	0.8 ± 0.4 (10)
Rice (2002)[b]	79.1	79.7	0.6 ± 0.9 (10)
Sands (2002)			
High phytate P diet	75.7	75.3	-0.4 ± 1.4 (10)
Low phytate P diet	74.0	74.5	0.4 ± 1.2 (10)
This study (2004)			
Corn-soybean meal diet	80.3	81.0	0.7 ± 0.5 (9)
Wheat-soybean meal diet	85.5	86.0	0.5 ± 0.4 (9)
Wheat-soybean meal-canola meal diet	77.7	80.6	2.9 ± 0.4 (9)
Barley-peas-canola meal diet	79.1	79.6	0.5 ± 0.6 (9)

[a] Differences are presented as percentage units ± SD; n = number of indispensable amino acids analyzed.

[b] Cited from Adeola and Sands (2003).

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CHAPTER IV. EFFECT OF PHYTASE SUPPLEMENTATION ON THE UTILIZATION OF SELECTED MINERALS IN HIGH- AND LOW- PHYTATE DIETS FED TO GROWING PIGS

A. Introduction

It has been recognized that supplementation of microbial phytase to swine diets improves the utilization of phosphorus (**P**) in feed ingredients of plant origin (Simons et al., 1990; Cromwell et al., 1993; Lei et al., 1993b, c). The effect of phytase supplementation on the utilization of other minerals, such as calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), and zinc (Zn) has been contradictory (Näsi, 1990; Pallauf et al., 1992; 1994; Lei et al., 1993a; Näsi and Helander, 1994; Adeola et al., 1995; Ashida et al. 1999).

It was hypothesized that the magnitudes of increase in the apparent fecal digestibilities (**AFD**) or other parameters of utilization of P, Ca and other minerals upon phytase supplementation are dependent on the initial phytate content and the activity of intrinsic phytase in the diet (Liao et al., 2002; 2003). In other words, relatively larger improvements would be expected when the content of phytate is high and the intrinsic phytase activity is low than when the content of phytate is low and the intrinsic phytase activity is high in the diet. To test this hypothesis, two model-type diets, relatively high and low in phytate content, were designed. The high-phytate diet was created by formulating a diet that contained 20% rice bran. Rice bran has a high phytate content and a low activity of intrinsic phytase (Liao et al., 2002).

The main objective of this study was to investigate the effect of phytase supplementation on P and Ca digestibility and balance and the AFD of Cu, Fe, Mg, Mn, Mo, and Zn in growing pigs fed the high- and low-phytate diets. The apparent ileal digestibilities (**AID**) and AFD of ash and dry matter (**DM**) were also determined.

B. Experimental Procedures

Dietary Treatments

Two basal diets were formulated to contain a high and a low concentration of phytate-P (Table IV-1). The high-phytate diet contained 20% of rice bran which is a rich source of phytate-P. To each basal diet, *Aspergillus niger* phytase (Natuphos®, DSM Food Specialties, Delft, The Netherlands) was supplemented at a rate of 1,000 phytase units (**FTU**) per kilogram to formulate two more experimental diets. One FTU is defined as the quantity of phytase that liberates 1 mmol of ortho-phosphate per minute from 5.1 mM Na-phytate at pH 5.5 and 37°C (Engelen et al., 2001). The diets were supplemented with inorganic P to meet the NRC (1998) standard for available P, which is 0.23% for growing pigs. Canola oil was included in the diets to increase the content of digestible energy up to the level recommended by NRC (1998). Vitamins and minerals were supplemented to meet the NRC (1998) standards. Free lysine was supplemented to fulfill the NRC (1998) standards. Chromic oxide was included in the diet at a rate of 0.25% as the digestibility indicator.

Prior to surgery and during the recuperation period, the pigs were fed *ad libitum* an 18% CP grower diet. Water was freely available from a low-pressure drinking nipple. The diets were fed in mash form. During the time the experimental diets were fed, water was added to the feed at a ratio of 2.5 to 1.

Animal Trial Procedures

Eight Genex F2 barrows (Large white × Landrace), average initial BW 25.3 kg, were obtained from the University of Alberta Swine Research and Technology Center. The barrows were housed individually in stainless steel metabolic crates (height: 85 cm; length: 140 cm; width: 65 cm) in a barn in which the temperature was maintained between 20 and 22°C. Following a 14-d adjustment period to the metabolic crates, each barrow was fitted with a simple T-cannula at the distal ileum, about 5 cm from the ileo-cecal sphincter. The preparation of the cannulas was previously described by Sauer et al.

(1983) and modified by De Lange et al. (1989). The surgical procedure was adapted from the procedure described by Sauer et al. (1983). A detailed description of pre- and post-operative care of animals was previously given by Sauer (1983) and Li et al. (1993). In general, intact pigs are used for the study of fecal digestibilities of minerals. However, in this trial cannulated pigs were used for reasons that these pigs were also used for determination of the ileal digestibilities of CP and AA (Chapter V). It needs to be pointed out here that cannulation of pigs does not affect fecal digestibility figures (Sauer and Ozimek, 1986).

Following a 7-d recuperation period after surgery, the barrows were fed the four experimental diets according to a repeated 4 × 4 Latin square design (n = 8). Each of the four experimental periods comprised 14 d. The diets were fed to the pigs at a rate of 2.4 times the maintenance requirement for ME (i.e., 100 kcal/kg of $BW^{0.75}$), based on the average BW of the pigs which was determined at the initiation of each experimental period. The daily meal allowances were offered twice daily at 0800 and 2000, equal amounts each meal.

The experimental proposal, surgical procedures, and procedures for use, care and treatment of the pigs were reviewed and approved by the Faculty of Agriculture, Forestry and Home Economics Animal Care Committee of the University of Alberta in accordance with the guidelines of CCAC (1993).

Sample Collection and Chemical Analysis

Samples of the feed ingredients were taken after the ingredients were ground through a 2-mm mesh screen. Samples of the diets were taken during the time the meal allowances were prepared. The collection of feces and urine was initiated at 0800 on d 8 of each experimental period and continued for 96 consecutive hours. Feces were frozen at –28°C immediately after collection. Urine, collected through glass wool, was measured volumetrically and stored at –4°C immediately. At the end of each experimental period, urine was pooled for each pig. During pooling the urine was filtered through triple layers of medical gauze. Then, an aliquot of 25% was taken and frozen at –28°C. Ileal digesta were collected into a soft plastic tube (length, 20 cm; i.d., 4 cm) for 36 h; from 0800 to 2000 on d 12, 13, and 14, respectively. Prior to collection, 8 mL 10%

(vol/vol) formic acid solution was placed into each tube. The tube was attached to the barrel of the cannula with a rubber band, and replaced as soon as it was nearly filled with digesta. Digesta were frozen at –28°C immediately after collection. Detailed procedures for collection of ileal digesta were previously described by Sauer (1983) and Li et al. (1993).

Prior to chemical analysis, fecal and also digesta samples were pooled leaving one sub-sample for each pig in each experimental period. Feces were dried in a forced-draft oven at 60°C until constant weight, and digesta were freeze-dried. The dried samples of feces and digesta, and samples of ingredients and diets were ground through a 0.5-mm mesh screen in a Thomas-Wiley Laboratory Mill (Arther H. Thomas Co., Philadelphia, PA, USA). Urine samples were filtered through Whatman #2 filter paper and then dried in a forced-draft oven prior to mineral analysis.

Dry matter was measured according to AOAC International (2000) official method 930.15. Ash was measured according to AOAC International (2000) official method 942.05. Gross energy was determined with an AC-300 Leco Automatic Calorimeter (Leco® Corporation, St. Joseph, MI, USA). Crude protein (N × 6.25) was measured with a Leco FP-428 Nitrogen Determinator. Total P content was determined photometrically by the molybdovanadate procedure according to AOAC International (2000) official method 965.17. The phytate P contents in the basal diets were analyzed according to the procedures described by Haug and Lantzsch (1983). Calcium, Mg, Fe, Mn, Cu and Zn were analyzed with an atomic absorption spectrophotometric procedure according to AOAC International (2000) official method 968.08. For Ca determination, lanthanum chloride was included at the final dilution step providing 1% (g/ml) lanthanum to minimize interference from other minerals. Molybdenum was analyzed with inductively coupled plasma spectroscopic methods. The intrinsic phytase activities (FTU/kg diet) were analyzed with a colorimetric enzymatic procedure according to AOAC International official method 2000.12 (Engelen et al., 1994, 2001). Chromic oxide was determined with a spectrophotometric procedure according to Fenton and Fenton (1979). Analyses of ingredients and diets were carried out in triplicate; analyses of digesta, urine and feces in duplicate.

Digestibility Calculations and Statistical Analysis

The AID and the AFD were determined for P, Ca, ash and DM, in addition to the AFD of Cu, Fe, Mg, Mn, Mo, and Zn. Phosphorus and Ca balances were also determined. The digestibilities were calculated by using the following equation:

$$D_D = 100\% - [(A_F \times I_D) / (A_D \times I_F)] \times 100\%$$

where D_D is the apparent digestibility of a parameter in the assay diet (%); A_F is the concentration of a parameter in ileal digesta or feces (%); I_D is the indicator concentration in the assay diet (%); A_D is the concentration of a parameter in the assay diet (%), I_F is the indicator concentration in ileal digesta or feces (%).

Based on the following linear model, the digestibility and balance values were subjected to statistical analysis by using the General Linear Model (GLM) Procedure of SAS® (1990).

$$Y_{ijk} = \mu + T_i + P_j + A_k + \varepsilon_{ijk}$$

where Y_{ijk} is a digestibility or balance value; μ is the overall mean of the digestibility or balance values; T_i is the fixed effect of dietary treatments and i = 1, 2, 3, 4; P_j is the random effect of experiment periods and j = 1, 2, 3, 4; A_k is the random effect of animals and k = 1, 2, 3, 4, 5, 6, 7, 8; ε_{ijk} is the residual experimental error with N $(0, \sigma^2)$. After analysis of variance, the means of the four treatments were compared by the Student-Newman-Keuls multiple range test. The effect of diet and the effect of dietary phytase supplementation were tested by orthogonal contrasts. Probability levels of $P \leq 0.05$ and $0.05 < P \leq 0.10$ were defined as significant differences and tendencies, respectively. The magnitudes of changes in each parameter upon phytase supplementation to the high- and low-phytate diets were compared with the Student's *t*-test at $P \leq 0.10$ and 0.05.

C. Results

Diet Analysis and Animal Health

The chemical compositions of the experimental diets are presented in Table IV-2. The analyzed values of the nutrients in the diets were close to the values calculated based on the analyzed values in the ingredients. As was expected, since rice bran has a relatively high content of P, neutral detergent fiber (**NDF**), and ash (Kaufmann, 2003), the contents of P, NDF, and ash were higher in the high- than in the low-phytate diets. As was intended, the content of phytate-P was higher in the high-phytate (0.48%) than in the low-phytate (0.22%) diets. The differences in phytase activities between the non- and phytase-supplemented diets were more than 1,000 FTU/kg, indicating that the batch of Natuphos® product used in the study contained more FTU than was declared.

All pigs remained healthy and usually consumed their meal allowances within 30 min after feeding throughout the experiment. The average BW of the pigs were 40.6, 44.4, 53.1, and 60.8 kg at beginning of experimental periods 1, 2, 3, and 4. The average BW of the pigs was 69.8 kg at conclusion of the experiment. The ADG of the pigs during the experiment was 520 g/d. Postmortem examinations conducted at the conclusion of the experiment revealed no intestinal adhesions or any other abnormalities.

Phosphorus Balance Study

As was expected, the AID and AFD of P were lower ($P < 0.001$) in the high- than in the low-phytate diets (Table IV-3). Without phytase supplementation, the AID and AFD of P in the high-phytate diet were 12.1 and 12.5 pu lower ($P < 0.01$), respectively, than in the low-phytate diet. With phytase supplementation, the AID and AFD of P in the high-phytate diet were 20.5 and 20.7 pu lower ($P < 0.01$), respectively, than in the low-phytate diet. Accordingly, the daily ileal and fecal outputs of P were higher ($P < 0.01$) in pigs fed the high-phytate diet than in pigs fed the low-phytate diet. There were no differences ($P > 0.10$) in the daily urinary outputs of P between the non-supplemented high- and low-phytate diets and between the phytase-supplemented high- and low-phytate diets. Also, there were no differences ($P > 0.10$) in the daily absorption and retention of

P between the non-supplemented high- and low-phytate diets and between the phytase-supplemented high- and low-phytate diets.

Phytase supplementation to both diets increased the AID and AFD of P (Table IV-3). The AID of P in the high- and low-phytate diets were increased ($P < 0.01$) by 10.5 and 18.9 pu, respectively. The AFD of P were increased ($P < 0.01$) by 11.4 and 19.6 pu in the high- and the low-phytate diets, respectively, which means that the fecal output of P was reduced ($P < 0.05$) by 14.9 and 30.5%, respectively. Obviously, the magnitudes of increase in the AID or AFD of P, or of a decrease in the fecal output of P, were not larger when phytase was supplemented to the high- compared to the low-phytate diet. The Student's *t*-test showed that there were no differences ($P > 0.10$) in the magnitudes of increase or decrease in the AFD or the fecal output of P between the high- and low-phytate diets upon phytase supplementation. The magnitudes of increase in the AID of P in the high-phytate diet even tended to be lower ($P = 0.09$) than in the low-phytate diet.

The supplementation of phytase to the high- and low-phytate diets increased ($P < 0.05$) the absorption of P by 1.17 and 1.34 g/d, respectively (Table IV-3). The magnitude of increase in P absorption upon phytase supplementation was not larger with the high- compared to the low-phytate diet. The daily urinary output of P increased ($P = 0.03$) after phytase supplementation to the high- and low-phytate diets diets, which may be caused by the increases in the daily P absorption. The magnitude of increase upon phytase supplementation was not larger ($P > 0.10$) with the high- than with the low-phytate diet. There were still increases in the daily retention of P upon phytase supplementation. The daily retention of P increased ($P < 0.05$) by 1.16 g (44.4%) and 1.32 g (50.2%) upon phytase supplementation to the high- and the low-phytate diet, respectively. It should be pointed out that the output of P in urine is of a very small magnitude compared to the output in feces. The magnitude of increase in the daily P retention upon phytase supplementation was similar ($P > 0.10$) for the high- and low-phytate diets.

These studies also showed that there is a net absorption of P in the large intestine (Table IV-3). For the non- and phytase-supplemented high-phytate diets, the differences between the daily ileal and fecal outputs of P were 0.76 and 0.83 g, respectively. In the same order for the diets, these values represent 28.9 and 21.8% of the total amount of P

absorbed. For the non- and phytase-supplemented low-phytate diets, the differences between the daily ileal and fecal outputs of P were 0.55 and 0.59 g, respectively. In the same order for the diets, these values represent 20.8 and 14.8% of the total amount of P absorbed.

Calcium Balance Study

The AID and AFD of Ca were lower ($P < 0.001$) in the high- than in the low-phytate diets (Table IV-3). Without phytase supplementation, the AID and AFD of Ca in the high-phytate diet were 14.6 and 20.0 pu lower ($P < 0.01$), respectively, than in the low-phytate diet. With phytase supplementation, the AID and AFD of Ca in the high-phytate diet were 22.7 and 25.4 pu lower ($P < 0.01$), respectively, than in the low-phytate diet. Accordingly, the daily ileal and fecal outputs of Ca were higher ($P < 0.001$) in pigs fed the high-phytate than in pigs fed the low-phytate diet. There were no differences ($P > 0.10$) in the daily urinary output, absorption, and retention of Ca between the non-supplemented high- and low-phytate diets and between the phytase-supplemented high- and low-phytate diets

Phytase supplementation to the low-phytate diet increased ($P < 0.05$) the AID and AFD of Ca, but not ($P > 0.10$) when supplemented to the high-phytate diet (Table IV-3). Supplementation to the low-phytate diet increased ($P < 0.05$) the AID of Ca by 6.5 pu, and the AFD by 6.9 pu. Obviously, the magnitudes of increase upon phytase supplementation in the AID or AFD of Ca were not larger with the high- than with the low-phytate diet. On the contrary, the magnitudes of increase in the AID and AFD of Ca were higher ($P < 0.05$) for the low- than for the high-phytate diet. The reduction in the daily fecal output of Ca, however, did not reach significance ($P > 0.15$).

There was a trend towards an increase ($P = 0.087$) in the daily absorption of Ca upon phytase supplementation to both diets (Table IV-3). The magnitude of the increase in Ca absorption upon phytase supplementation to the high-phytate diet was not larger ($P > 0.10$) than to the low-phytate diet. The daily urinary outputs of Ca decreased ($P < 0.001$) after phytase supplementation to either diet. The magnitudes of the decrease were not different ($P > 0.10$) between the two diets. There was an increase ($P < 0.05$) in Ca

retention upon phytase supplementation to the diets. The magnitudes of the increases in Ca retention were not different ($P > 0.10$) between the high- and the low-phytate diets.

There was some net absorption of Ca in the large intestine when the low-phytate diet was fed (Table IV-3). For the non- and phytase-supplemented low-phytate diets, the differences between the daily ileal and fecal outputs of Ca were 0.39 and 0.40 g, respectively. In the same order for the diets, these values represent 5.9 and 5.4% of the total amount of Ca absorbed. For the non- and phytase-supplemented high-phytate diets, the differences between the daily ileal and fecal outputs of Ca were -0.38 g (net appearance) and 0.17 g, respectively.

Digestibilities of Other Minerals

The AFD of Mg was lower ($P < 0.05$) in the high- than in the low-phytate diets (Table IV-4). There were no differences ($P > 0.10$) in the AFD of Fe and Mo between the high- and the low-phytate diets. The AFD of Cu, Mn, and Zn were higher ($P < 0.01$) in the high- than in the low-phytate diets.

Phytase supplementation had no effect ($P > 0.10$) on the AFD of Cu, Fe, Mg, Mn, and Mo (Table IV-4). Supplementation of phytase to the high-phytate diet decreased ($P < 0.05$) the AFD of Zn, but not ($P > 0.10$) to the low-phytate diet. The Student's *t*-test showed that there were no differences ($P > 0.10$) in the magnitudes of decrease in the AFD of Zn between the high- and low-phytate diets upon phytase supplementation.

Digestibilities of Ash and Dry matter

There were no differences ($P > 0.10$) in the AID of ash between the high- and the low-phytate diets (Table IV-5). The AFD of ash was 10.5 or 14.8 pu lower ($P < 0.01$) in the high- than in the low-phytate diet without or with phytase supplementation, respectively.

There was no effect ($P > 0.10$) of phytase supplementation to the high- and low-phytate diets on the AID of ash (Table IV-5). Phytase supplementation to the low-phytate diet increased ($P < 0.05$) the AFD of ash by 6.4 pu. There was a trend ($P < 0.10$) towards an increase when phytase was supplemented to the high-phytate diet. The magnitudes of increase in the AFD and also in the AID of ash in the high-phytate diet

were not larger than in the low-phytate diet. The Student's *t*-test showed that there were no differences ($P < 0.10$) in the magnitudes of increase in the AFD and in the AID of ash between the high- and the low-phytate diet upon phytase supplementation.

Without phytase supplementation, the AID and AFD of DM were 7.8 and 7.1 pu lower ($P < 0.05$) in the high- than in the low-phytate diet, respectively (Table IV-5). With phytase supplementation, the AID and AFD of DM were 9.0 and 7.4 pu lower ($P < 0.05$) in the high- than in the low-phytate diet, respectively. Phytase supplementation to either diet did not ($P > 0.10$) result in an improvement in the AID or the AFD of DM.

D. Discussion

The AID and AFD of P, Ca, ash, and DM were lower ($P < 0.001$) in the high- than in the low-phytate diet (Tables IV-3 and IV-5). The AFD of Cu, Mn, and Zn were higher ($P < 0.001$) in the high- than in the low-phytate diet (Table IV-4). There were no differences ($P > 0.10$) in the AFD of other minerals between the diets. The dietary effects on the utilization of different minerals must be associated with the different levels of inclusion of rice bran and other ingredients including inorganic supplements. Although it is not clear why the dietary effects on these minerals are different, it is known that there are many factors and interactions among the dietary components including the contents of phytate/phytic acid, fibre, and minerals, which all play a role in influencing the digestibilities of different minerals.

Commercial phytase was initially developed to increase the bioavailability of phytate-P in feedstuffs of plant origin for nonruminants in order to reduce the animal reliance on inorganic P supplementation, and decrease the P output in manure. Previous investigations, mainly with corn-soybean meal diets fed to growing/finishing pigs without inorganic P supplementation, have shown that supplementation of phytase can achieve these goals (Simons et al., 1990; Näsi, 1990; Jongbloed et al., 1992; Cromwell et al., 1993, 1995; Mroz et al., 1994; Kemme et al., 1999). However, in light of current knowledge, it still seems not possible to exceed P availabilities of 60 to 70% in feed ingredients of plant origin, even if phytase is supplemented at a very high rate, which

indicates that there are some key research areas remaining that need to be investigated for the practice of phytase supplementation.

Phytase supplementation to both the high- and the low-phytate diets increased the AID and also the AFD of P ($P < 0.001$), which is in agreement with nearly all previous studies (Kornegay, 1996; 1999; 2001). However, the magnitude of increase upon phytase supplementation in either the AID or the AFD of P in the high-phytate diet was not larger than in the low-phytate diet. These results do not support those reported by Sands (2002) and the hypothesis stated for this study, suggesting that there must be other factors and/or interactions within the high-phytate diet that affect the action of phytase and that a new phytase enzyme with a high efficacy needs to be developed. Further studies on these factors and the development of a new phytase enzyme are warranted.

The daily outputs of P in feces upon phytase supplementation were reduced by 14.9 and 30.5% for the high- and the low-phytate diets, respectively (Table IV-3). As was reported in Chapter II, the daily output of P in urine was of a very small magnitude compared to in feces, irrespective of phytase supplementation. The absorption and rentention of P, therefore, were increased along with the improvements in the AID and AFD, especially for the low-phytate diet. In agreement with the results reported in Chapter II, the improvement in P retention implies that the standard for bioavailable P for growing pigs, as suggested by NRC (1998), namely 0.23%, may be too low.

Phytase supplementation increased the AID and also the AFD of Ca in the low-phytate diet ($P < 0.05$), but not in the high-phytate diet (Table IV-3). Contrary to the hypothesis stated for this study, the magnitude of increase in either the AID or the AFD of Ca upon phytase supplementation to the high-phytate diet was obviously not larger than to the low-phytate diet, which indicates that some other dietary factors play a role in influencing the AID and AFD of Ca. The dietary Ca level was 0.85% in the low-phytate diet, and was 1.31% in the high-phytate diet (Table IV-2). Jongbloed et al. (1993) reported the largest increase in the AFD of Ca from the diet with the lowest Ca content and the highest rate of phytase supplementation, which is in agreement with the results of this study.

This study is the first to report that the daily urinary output of Ca was decreased ($P < 0.001$) after phytase supplementation. The retention of Ca was therefore increased,

since the AFD of Ca was increased and the fecal and urinary outputs were decreased (Table IV-3). In general, the results obtained in this study regarding the effect of phytase on Ca utilization support our previous results reported in Chapter II.

The AFD of Cu, Fe, Mg, Mn, Mo, and Zn were also determined in this study. The results showed that there were no effects of phytase supplementation on the AFD of these minerals, except for Zn (Table IV-4). It should be stressed that the sources of these minerals included both the feed ingredients and the inorganic supplements (Table IV-1). The effect of phytase supplementation on the AFD of these minerals did not directly correlate with the AFD of P or Ca. Changes in the AFD of these minerals upon phytase supplementation can not be simply predicted from the changes in the AFD of P or Ca. Negative values of the AFD of Cu, Fe, Mn, and Zn were obtained in this study, which is in agreement with the results reported in Chapter II. One explanation for this may be that undigested phytate/phytic acid or other components still bind considerable amounts of these minerals. Supplementation (via the premix) of more inorganic minerals than the requirements of growing pigs (Table IV-2), and the unknown interactions among these minerals (Adeola et al., 1995), may also explain the very low AFD of these minerals.

Phytase supplementation increased the AFD, but not the AID of ash. This can be explained by the fact that large amounts of minerals, especially Cu, Fe, Na, and Mn were secreted into the lumen of the small intestine (data not shown), and reabsorbed in the large intestine. There were no differences in the AID and also in the AFD of DM between the high- and the low-phytate diets. These results are in agreement with previous results reported in Chapter II. The AFD of DM depends not only on the AFD of ash but also on the AFD of organic matter. The changes in the AFD of ash were not large enough to affect the AFD of DM.

E. Implications

The AID and/or the AFD of the majority of minerals, ash, and DM were lower in the high- than in the low-phytate diet. Phytase supplementation increased the AID and the AFD of P and Ca, but decreased the AFD of Zn. There was no effect of phytase supplementation on the AFD of other minerals. The daily retention of both P and Ca was

increased upon phytase supplementation to the diets. Phytase supplementation to the high-phytate diet did not result in a larger increase in the AID or in the AFD of minerals, ash, and DM compared to low-phytate diet.

Table IV-1. Formulation (%) of the experimental diets[a]

Phytate level	High-phytate		Low-phytate	
Phytase (FTU/kg)	0	1000	0	1000
Corn	29.49	29.49	32.12	32.12
Corn starch	-	-	15.00	15.00
Rice bran	20.00	20.00	2.00	2.00
Barley	15.00	15.00	16.00	16.00
Wheat	5.00	5.00	5.00	5.00
Soybean meal	19.00	19.00	25.00	25.00
Canola meal	5.00	5.00	2.00	2.00
Canola oil	4.20	4.20	0.40	0.40
Mono-dicalcium phosphate[b]	0.33	0.33	0.60	0.60
Limestone	1.10	1.10	1.02	1.02
Salt	0.35	0.35	0.36	0.36
L-lysine-HCl	0.04	0.04	-	-
Choline chloride[c]	0.05	0.05	0.05	0.05
Vitamin premix[d]	0.10	0.10	0.10	0.10
Mineral premix[d]	0.10	0.10	0.10	0.10
Chromic oxide	0.25	0.25	0.25	0.25

[a] As-fed basis.

[b] Contained P, 21.0%; Ca, 15.0%; F, 2.1 g/kg, and Fe, 9.0 g/kg; Supplied by Champion Feed Services Ltd, Westlock, AB, Canada.

[c] Contained 60% Choline chloride; Supplied by Champion Feed Services Ltd, Westlock, AB, Canada.

[d] Supplied by Champion Feed Services Ltd, Westlock, AB, Canada. The mineral premix provided (per kilogram of diet): Fe, 135 mg; Zn, 135 mg; Mn, 40 mg; Cu, 20 mg; I, 0.5 mg; Co, 0.5 mg, and Se, 0.3 mg. The vitamin premix provided (per kilogram of diet): vitamin A, 12,000 IU; vitamin D_3, 1,500 IU; vitamin E, 50 IU; vitamin K_3, 1.5 mg; riboflavin, 5.5 mg; niacin, 25 mg; pantothenic acid, 15 mg, and vitamin B_{12}, 0.02 mg.

Table IV-2. Chemical compositions of the experimental diets[a]

Phytate level	High-phytate		Low-phytate	
Phytase (FTU/kg)	0	1000	0	1000
Dry matter (%)	89.5	89.6	88.2	88.3
Gross energy (kcal/kg)	4,232	4,240	3,924	3,940
Metabolizable energy (kcal/kg)[b]	3,286	3,286	3,288	3,288
Crude protein (%)	18.73	18.77	18.10	17.82
Neutral detergent fiber (%)	26.4	26.2	19.6	19.4
Lysine (%)	0.98	0.96	0.91	0.90
Ash (%)	7.23	7.14	5.23	5.20
Macro-minerals (%)				
Phosphorus (total)	0.77	0.77	0.51	0.52
Phosphorus (available)[b]	0.23	0.23	0.23	0.23
Calcium	1.30	1.31	0.86	0.83
Magnesium	0.35	0.35	0.17	0.17
Micro-minerals (ppm)				
Copper	24.3	24.3	13.0	13.2
Iron	229	280	324	272
Manganese	131	125	70.2	69.9
Molybdenum	1.6	1.1	1.6	1.3
Zinc	211	194	167	196
Phytate-phosphorus (%)	0.478	0.483	0.221	0.224
Phytase (FTU/kg diet)	136	1740	115	1910

[a] As-fed basis.

[b] Calculated, based on NRC (1998).

Table IV-3. Effect of phytase supplementation to a high- and a low-phytate diet on phosphorus and calcium balance in growing pigs

Phytate level	High-phytate		Low-phytate			*P*-value[b]	
Phytase (FTU/kg)	0	1000	0	1000	SEM[a]	Dt-Ef[c]	Ps-Ef[d]
Phosphorus							
Intake (g/d)	10.49	10.49	7.01	7.01	0.073		
Ileal digestibility (%)	17.9[g]	28.4[f]	30.0[f]	48.9[e]	1.590	<0.0001	<0.0001
Ileal output (g/d)	8.62[e]	7.52[f]	4.91[g]	3.62[h]	0.157	<0.0001	<0.0001
Fecal digestibility (%)	25.5[g]	36.9[f]	38.0[f]	57.6[e]	1.228	<0.0001	<0.0001
Fecal output (g/d)	7.86[e]	6.69[f]	4.36[g]	3.03[h]	0.153	<0.0001	<0.0001
Urinary output (mg/d)	15.87	26.58	14.83	28.77	5.391	0.9164	0.0346
Absorbed (g/d)	2.63[f]	3.80[e]	2.64[f]	3.98[e]	0.114	0.3938	<0.0001
Retained (g/d)	2.61[f]	3.77[e]	2.63[f]	3.95[e]	0.116	0.4045	<0.0001
Calcium							
Intake (g/d)	17.80	17.80	11.53	11.53	0.131		
Ileal digestibility (%)	39.5[g]	37.9[g]	54.1[f]	60.6[e]	1.438	<0.0001	0.1081
Ileal output (g/d)	10.80[e]	11.12[e]	5.34[f]	4.59[f]	0.284	<0.0001	0.4533
Fecal digestibility (%)	37.5[g]	39.0[g]	57.5[f]	64.4[e]	1.632	<0.0001	0.0184
Fecal output (g/d)	11.18[e]	10.95[e]	4.95[f]	4.19[f]	0.343	<0.0001	0.1639
Urinary output (mg/d)	403.0[e]	158.2[f]	368.8[e]	47.45[f]	53.87	0.1951	<0.0001
Absorbed (g/d)	6.62	6.85	6.57	7.34	0.275	0.4357	0.0874
Retained (g/d)	6.22	6.69	6.20	7.29	0.289	0.3273	0.0149

[a] Standard error of the mean (n = 8).

[b] *P*-values obtained from the orthogonal contrasts.

[c] Effect of diet.

[d] Effect of phytase supplementation.

[e, f, g, h] Means within rows with different superscripts differ ($P < 0.05$).

Table IV-4. Effect of phytase supplementation to a high- and a low-phytate diet on the apparent fecal digestibilities (%) of copper, iron, magnesium, manganese, molybdenum, and zinc in growing pigs

Phytate level	High-phytate		Low-phytate			*P*-value[b]	
Phytase (FTU/kg)	0	1000	0	1000	SEM[a]	Dt-Ef[c]	Ps-Ef[d]
Copper	8.9[e]	7.5[e]	-83.1[f]	-76.3[f]	2.538	<0.0001	0.3034
Iron	-18.4	-20.6	-3.3	-7.4	9.241	0.1437	0.7343
Magnesium	26.6	26.5	28.5	31.8	1.345	0.0145	0.2364
Manganese	6.0[e]	3.8[f]	-4.3[g]	-4.3[g]	0.645	<0.0001	0.1016
Molybdenum	66.2	64.1	67.0	68.5	2.492	0.3611	0.9953
Zinc	2.5[e]	-2.8[f]	-11.8[g]	-16.8[g]	1.412	<0.0001	0.0063

[a] Standard error of the mean (n = 8).

[b] *P*-values obtained from the orthogonal contrasts.

[c] Effect of diet.

[d] Effect of phytase supplementation.

[e, f, g] Means within rows with different superscripts differ ($P < 0.05$).

Table IV-5. Effect of phytase supplementation to a high- and a low-phytate diet on the apparent ileal and fecal digestibilities (%) of ash and dry matter in growing pigs

Phytate level	High-phytate		Low-phytate			*P*-value[b]	
Phytase (FTU/kg)	0	1000	0	1000	SEM[a]	Dt-Ef[c]	Ps-Ef[d]
Ash							
Ileal digestibility	17.8	18.1	17.2	22.1	1.713	0.3290	0.1508
Fecal digestibility	42.8[g]	44.9[g]	53.3[f]	59.7[e]	0.753	<0.0001	<0.0001
Dry matter							
Ileal digestibility	61.4[f]	60.0[f]	69.2[e]	69.0[e]	0.622	<0.0001	0.1855
Fecal digestibility	81.4[f]	81.1[f]	88.5[e]	88.5[e]	0.299	<0.0001	0.6395

[a] Standard error of the mean (n = 8).

[b] *P*-values obtained from the orthogonal contrasts.

[c] Effect of diet.

[d] Effect of phytase supplementation.

[e, f, g] Means within rows with different superscripts differ ($P < 0.05$).

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CHAPTER V. EFFECT OF PHYTASE SUPPLEMENTATION ON THE DIGESTIBILITIES OF CRUDE PROTEIN, AMINO ACIDS AND ENERGY IN HIGH- AND LOW-PHYTATE DIETS FED TO GROWING PIGS [1]

A. Introduction

It has been recognized that supplementation of microbial phytase to swine diets improves the absorption and utilization of phosphorus (**P**) in feed ingredients of plant origin (Simons et al., 1990; Cromwell et al., 1993; Lei et al., 1993a, b). At present, there is a lot of interest in the effect of phytase supplementation on the utilization of crude protein (**CP**) and amino acids (**AA**). Some studies have shown no effect of phytase supplementation on the the apparent ileal digestibilities (**AID**) of CP and AA (e.g., Nasi et al., 1995; Yi et al., 1996; Valaja et al., 1998), while in other studies phytase supplementation improved the AID of CP and AA (e.g., Officer and Batterham, 1992a; Mroz et al., 1994; Kornegay et al., 1998; Kemme et al., 1999).

Liao et al. (2002) hypothesized that a response in the AID of CP and AA to phytase supplementation is dependent on the initial phytate content and the activity of intrinsic phytase in the diet. In other words, a positive response in the AID of CP and AA to phytase supplementation is more likely to occur when the content of phytate is high and the intrinsic phytase activity is low than when the content of phytate is low and the intrinsic phytase activity is high in the diet.

The aforementioned hypothesis was tested in this study. Two model diets, relatively high and low in phytate-P, were formulated. The high-phytate diet was created by formulating a diet that contained 20% rice bran. Rice bran has a high phytate content and a low activity of intrinsic phytase (Liao et al., 2002).

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[1] Portions of this study were presented at 2004 Banff Pork Seminar, Banff, AB, Canada.

The main objective of this study was to investigate the effect of phytase supplementation to high- and low-phytate diets on the AID of CP, AA and energy, and the apparent fecal digestibilities (**AFD**) of CP and energy with growing pigs.

B. Experimental Procedures

Dietary Treatments

Two basal diets were formulated to contain a high and a low concentration of phytate-P (Table V-1). The high-phytate diet contained 20% of rice bran which is a rich source of phytate-P. To each basal diet, *Aspergillus niger* phytase (Natuphos®, DSM Food Specialties, Delft, The Netherlands) was supplemented at a rate of 1,000 phytase units (**FTU**) per kilogram to formulate two more experimental diets. One FTU is defined as the quantity of enzyme that liberates 1 mmol of ortho-phosphate per minute from 5.1 mM Na-phytate at pH 5.5 and 37°C (Engelen et al., 2001). The diets were supplemented with inorganic P to meet the NRC (1998) standards for available P, which is 0.23% for growing pigs. Canola oil was included in the diets to increase the content of digestible energy up to the level recommended by NRC (1998). Vitamins and minerals were supplemented to meet the NRC (1998) standards. Free lysine was supplemented to the diets to fulfill the NRC (1998) standards. Chromic oxide was included in the diet at a rate of 0.25% as the digestibility indicator.

Prior to surgery and during the recuperation period, the pigs were fed *ad libitum* an 18% CP grower diet. Water was freely available from a low-pressure drinking nipple. The diets were fed in mash form. During the time the experimental diets were fed, water was added to the feed at a ratio of 2.5 to 1.

Animal Trial Procedures

Eight Genex F2 barrows (Large white × Landrace), average initial BW 25.3 kg, were obtained from the University of Alberta Swine Research and Technology Center. The barrows were housed individually in stainless steel metabolic crates (height: 85 cm; length: 140 cm; width: 65 cm) in a barn in which the temperature was maintained

between 20 and 22°C. Following a 14-d adjustment period to the metabolic crates, each barrow was fitted with a simple T-cannula at the distal ileum, about 5 cm from the ileo-cecal sphincter. The preparation of the cannulas was previously described by Sauer et al. (1983) and modified by De Lange et al. (1989). The surgical procedure was adapted from the procedure described by Sauer et al. (1983). A detailed description of pre- and post-operative care of animals was previously given by Sauer (1983) and Li et al. (1993).

Following a 7-d recuperation period after surgery, the barrows were fed the four experimental diets according to a repeated 4 × 4 Latin square design (n = 8). Each experimental period comprised 14 d. The diets were fed to the pigs at a rate of 2.4 times the maintenance requirement for ME (i.e., 100 kcal/kg of $BW^{0.75}$), based on the average BW of the pigs which was determined at the initiation of each experimental period. The daily meal allowances were offered twice daily at 0800 and 2000, equal amounts each meal.

The experimental proposal, surgical procedures, and procedures for care and treatment of the pigs were reviewed and approved by the Faculty of Agriculture, Forestry and Home Economics Animal Care Committee of the University of Alberta in accordance with the guidelines of CCAC (1993).

Sample Collections and Chemical Analysis

Samples of the feed ingredients were taken after the ingredients were ground through a 2-mm mesh screen. Samples of the diets were taken during the time the meal allowances were prepared. The collection of feces was initiated at 0800 on d 8 of each experimental period and continued for 96 consecutive hours. Feces were frozen at –28°C immediately after collection. Ileal digesta were collected into a soft plastic tube (length, 20 cm; i.d., 4 cm) for 36 h; from 0800 to 2000 on d 12, 13, and 14. Prior to collection, 8 mL 10% (vol/vol) formic acid solution was placed into each tube. The tube was attached to the barrel of the cannula with a rubber band and replaced as soon as it was nearly filled with digesta. Digesta were frozen at –28°C immediately after collection. Detailed procedures for collection of ileal digesta were previously described by Sauer (1983) and Li et al. (1993).

Prior to chemical analysis, feces and also digesta were pooled leaving one sub-sample for each pig in each experimental period. Feces were dried in a forced-draft oven at 60°C until constant weight. Digesta were freeze-dried. The dried samples of feces and digesta, and samples of ingredients and diets were ground through a 0.5-mm mesh screen in a Thomas-Wiley Laboratory Mill (Arther H. Thomas Co., Philadelphia, PA, USA).

Dry matter was measured according to AOAC International (2000) official method 930.15. Gross energy was determined with an AC-300 Leco Automatic Calorimeter (Leco® Corporation, St. Joseph, MI, USA). Crude protein (N × 6.25) was measured with a Leco FP-428 Nitrogen Determinator. The phytate P contents in the diets were analyzed according to procedures described by Haug and Lantzsch (1983). The intrinsic phytase activities (FTU/kg diet) were analyzed with a colorimetric enzymatic procedure according to AOAC International official method 2000.12 (Engelen et al., 1994, 2001). Chromic oxide was determined with a spectrophotometric procedure according to Fenton and Fenton (1979). Analyses of ingredients and diets were carried out in triplicate; analyses of feces and digesta in duplicate.

For AA analyses, approximately 0.1 g of finely ground digesta (< 0.1 mm) was weighed into a screw-capped culture tube, mixed with 3 mL of 6 N HCl and hydrolyzed for 24 h in an oven at 110°C. The hydrolyzed samples were mixed with the internal standard, DL-amino-n-butyric acid, and centrifuged at 1,110 × g for 15 min at 4°C. The supernatant of the sample was analyzed using a Varian 5000 high performance liquid chromatography system with a reverse-phase column and a Varian Fluorichrom detector (Varian Canada Inc., Mississauga, ON) according to the principles outlined by Jones and Gilligan (1983). The AA were derivatized with an *o*-phthaldialdehyde reagent solution. The mobile phase consisted of two solvents (A and B) with a flow rate of 1.1 ml/min. Solvent A was a 0.1 M sodium acetate (pH 7.2) contained methanol and tetrahydrofuran in a ratio of 90 to 5. Solvent B was pure methanol. Peaks were recorded and integrated using the Ezchrom™ Chromatography Data System (version 4.2; Shimadzu Scientific Instruments Inc., Columbia, MD, USA). The procedure was described in detail by Sedgwick et al. (1991). Cysteine, proline, and tryptophan were not determined.

Digestibility Calculation and Statistical Analysis

The AID of energy, CP and AA and the AFD of energy and CP were calculated by using the following equation:

$$D_D = 100\% - [(A_F \times I_D) / (A_D \times I_F)] \times 100\%$$

where D_D is the AID or AFD of a parameter in the assay diet (%); A_F is the concentration of a parameter in ileal digesta or feces (%); I_D is the indicator concentration in the assay diet (%); A_D is the concentration of a parameter in the assay diet (%), I_F is the indicator concentration in ileal digesta or feces (%).

Based on the following linear model, the digestibility values were subjected to statistical analysis using the General Linear Model Procedure of SAS® (1990).

$$Y_{ijk} = \mu + T_i + P_j + A_k + \varepsilon_{ijk}$$

where Y_{ijk} is a digestibility value; μ is the overall mean of the digestibility values; T_i is the fixed effect of dietary treatments and i = 1, 2, 3, 4; P_j is the random effect of experimental periods and j = 1, 2, 3, 4; A_k is the random effect of animals and k = 1, 2, 3, 4, 5, 6, 7, 8; ε_{ijk} is the residual experimental error with N (0, σ^2). After analysis of variance, the means of the four treatments were compared by the Student-Newman-Keuls multiple range test. The effect of diet and the effect of dietary phytase supplementation were also tested by orthogonal contrasts. Probability levels of $P \leq 0.05$ and $0.05 < P \leq 0.10$ were defined as significant differences and tendencies toward differences, respectively. The magnitudes of parameter changes upon phytase supplementation to the high-phytate diet were compared to the low-phytate diet by the Student's t-test at $P \leq 0.10$ and 0.05.

C. Results

Diet Analysis and Animal Health

The chemical compositions of the experimental diets are presented in Table V-2. The analyzed values of the nutrients in the diets were close to the values calculated based on the analyzed values in the ingredients. As was expected, since rice bran has a

relatively high content of energy, neutral detergent fiber (**NDF**), P and ash (Kaufmann, 2003), the contents of these were higher in the high- than in the low-phytate diet. As was intended, the content of phytate-P was higher in the high-phytate (0.48%) than in the low-phytate (0.22%) diet. The differences in phytase activities between the phytase-supplemented and non-supplemented diets were more than 1,000 FTU/kg indicating that the batch of Natuphos® product used in the study contained more FTU than was declared.

All pigs remained healthy and usually consumed their meal allowances within 30 min after feeding throughout the experiment. The average BW of the pigs were 40.6, 44.4, 53.1, and 60.8 kg at beginning of experimental periods 1, 2, 3, and 4. The average BW of the pigs was 69.8 kg at conclusion of the experiment. The ADG of the pigs during the experiment was 520 g/d. Postmortem examinations conducted at the conclusion of the experiment revealed no intestinal adhesions or any other abnormalities.

Apparent Ileal Digestibilities of Energy, Crude Protein and Amino Acids

The AID of energy, CP, and AA were lower in the high- than in the low- phytate diet, irrespective of phytase supplementation (Table V-3). The differences were highly significant for energy ($P < 0.001$), CP ($P < 0.001$), and AA ($P < 0.05$) with the exception of glycine ($P > 0.10$). Without phytase supplementation, the AID of energy was 4.7 percentage units (**pu**) lower ($P < 0.05$) in the high- than in the low-phytate diet. The AID of CP and the average of the AID of the indispensable AA (excluding methionine) were 2.9 and 3.2 pu lower ($P < 0.05$) in the high-phytate diet. With phytase supplementation, the AID of energy was 5.9 pu lower ($P < 0.05$) in the high- than in the low-phytate diet. The AID of CP and the average of the AID of the indispensable AA (excluding methionine) were 3.4 and 3.1 pu lower ($P < 0.05$) in the high-phytate diet.

The supplementation of phytase had no significant effect ($P > 0.10$) on the AID of energy and AA (Table V-3). There was a trend ($P < 0.10$) towards increases in the AID of CP upon phytase supplementation. As in many other studies, there were usually small numerical increases in the AID of AA upon phytase supplementation. The average values for the AID of the indispensable (excluding methionine) and of the dispensable AA were increased by 0.6 and 1.3 pu, respectively, when phytase was supplemented to the high-phytate diet. The average values for the AID of the indispensable (excluding

methionine) and the dispensable AA increased by 0.6 and 0.7 pu, respectively, when phytase was supplemented to the low-phytate diet. The AID of CP increased by 0.6 and 1.1 pu in the high- and low-phytate diets, respectively. The Student's *t*-test showed that there was no difference ($P > 0.10$) in the magnitudes of increase in the AID of CP and AA between the high- and the low-phytate diet upon phytase supplementation.

Apparent Fecal Digestibilities of Energy and Crude Protein

Irrespective of phytase supplementation, the AFD of energy and CP were lower ($P < 0.05$) in the high- than in the low-phytate diets (Table V-4). The AFD of energy and of CP in the high-phytate diet were 5.2 and 2.7 pu, respectively, lower ($P < 0.05$) than in the low-phytate diet.

The supplementation of phytase to either the high- or low-phytate diet did not affect ($P > 0.10$) the AFD of energy and CP (Table V-4). Numerically, the AFD of CP increased by 0.6 and 0.2 pu ($P > 0.10$) in the high- and the low-phytate diet, respectively. The magnitude of increase of the AFD of CP in the high-phytate diet was numerically larger ($P > 0.10$) than when phytase was supplemented to the low-phytate diet. There was no increase in the AFD of energy upon phytase supplementation to either diet.

As was expected, the AFD of energy and CP were higher ($P > 0.05$) than their respective AID (Table V-4 vs. Table V-3). The differences between the AFD and the AID of energy and CP were 16.9 ± 0.41 and 12.8 ± 0.53 pu (mean ± SD, n = 4), respectively.

D. Discussion

As was discussed by Kies et al. (1997) and Selle et al. (2000), there are four mechanisms that can be suggested for a possible negative effect of the dietary phytate content on the AID of protein and AA: 1) inherent phytate-protein/AA complexes in feedstuffs, 2) *de novo* formation of phytate-protein complexes in the digestive tract, 3) *de novo* formation of phytate-AA complexes after digestion of dietary protein in the digestive tract, and 4) formation of complexes between phytate and proteolytic enzymes in the digestive tract. As was summarized by Adeola and Sands (2003), in theory the

formation of ternary complexes of phytate, cations and protein/AA during intestinal passage may have a negative effect on the digestibilities of protein, AA, and minerals, and protease activities. This process involving mineral chelation may also remove the cofactors required for the optimal activities of proteolytic enzymes.

There is a lack of consistency in response to phytase supplementation in the AID of CP and AA. As was pointed out by Kemme et al. (1999), there are many factors that should be considered, including the type of protein, the solubility of protein, *in situ* pH, the concentrations of other dietary minerals and three-way interactions between phytate, protein/AA, and proteolytic enzymes in the digestive tract. In some studies there was no effect of phytase supplementation on the AID of AA (Lei et al., 1997; Valaja et al., 1998; Rice, 2002; Sands, 2002). In other studies there was an effect of phytase supplementation on the AID of AA (Officer and Batterham, 1992a; Mroz et al., 1994; Kornegay et al., 1998; Kemme et al., 1999; Radcliffe et al., 1999; Zhang and Kornegay, 1999). With the exception of results reported by Officer and Batterham (1992a), the improvements in the AID of AA were of a small magnitude, in the order of 0.4 to 2.6 pu. These small increases reached significance ($P < 0.05$) for some of the indispensable AA in some of the studies. As was also discussed in Chapter III, some caution is warranted in the interpretation of the results by Officer and Batterham (1992a). First of all, the slaughter method was used to determine the AID of AA. This method for determining the AID of AA is open to criticism as was reviewed by Sauer (1976). Secondly, the publication by Officer and Batterham (1992a) and a related one (Officer and Batterham, 1992b) only provided very limited information on the experimental procedures that were employed.

As was suggested by Liao et al. (2002), the inconsistent responses in the AID of CP and AA to phytase supplementation can perhaps, in part, be attributed to the content of phytate and the activity of intrinsic phytase in the diet. The content of phytate-P and the activity of intrinsic phytase in the high-phytate diet were 0.48% and 136 FTU/kg, respectively (Table V-2). In the same order for the low-phytate diet these values were 0.22% and 115 FTU/kg, respectively. As was mentioned previously, the high-phytate diet was created by the inclusion of 20% rice bran which is a rich source of phytate-P (Liao et al., 2002).

As was expected, the AID of CP, AA, and also energy were lower in the high- than in the low-phytate diet. The differences were significant ($P < 0.05$) for energy, CP and the majority of the AA. The lower AID of CP, AA, and energy in the high-phytate diet are a direct result of the inclusion of 20% rice bran in the diet. As was shown by Kaufmann (2003), the AID of CP, AA, and energy are relatively low in rice bran. For example, the AID of CP and energy ranged from 38.3 to 67.3% and from 60.5 to 65.8%, respectively, in four samples of rice bran fed to growing pigs.

Supplementation of phytase to both the high- and low-phytate diets did not affect ($P > 0.05$ or 0.10) the AID of CP, AA, and energy (Table V-3). Also, the magnitudes of increases in the AID of CP, AA, and energy were not larger ($P > 0.10$) when phytase was supplemented to the high- compared to the low-phytate diet. These results suggest that a possible response in the AID of CP and AA to phytase supplementation is independent of the phytate-P content of the diet. These results are in agreement with those reported by Sands (2002), who also showed that microbial phytase supplementation to high- or low-phytin diets did not improve the AID of AA in growing pigs. Consistent with previous studies (Chapter III), there were small numerical increases in the AID of CP and AA upon phytase supplementation. Supplementation of phytase, as in previous studies (Chapter III), did not affect the AFD of energy and CP (Table V-4).

E. Implications

Supplementation of microbial phytase to the diets formulated to contain a relatively high and low content of phytate-P did not affect ($P > 0.10$) the AID of AA, although the AID of CP had a trend ($P < 0.10$) to be increased. These results suggest that a possible response in the AID of AA or CP to phytase supplementation is independent of the dietary phytate-P content. Consistent with many reports in the literature, there were small numerical increases in the AID of CP and AA upon phytase supplementation.

Table V-1. Formulation (%) of the experimental diets[a]

Phytate level	High-phytate		Low-phytate	
Phytase (FTU/kg)	0	1000	0	1000
Corn	29.49	29.49	32.12	32.12
Corn starch	-	-	15.00	15.00
Rice bran	20.00	20.00	2.00	2.00
Barley	15.00	15.00	16.00	16.00
Wheat	5.00	5.00	5.00	5.00
Soybean meal	19.00	19.00	25.00	25.00
Canola meal	5.00	5.00	2.00	2.00
Canola oil	4.20	4.20	0.40	0.40
Mono-dicalcium phosphate[b]	0.33	0.33	0.60	0.60
Limestone	1.10	1.10	1.02	1.02
Salt	0.35	0.35	0.36	0.36
L-lysine-HCl	0.04	0.04	-	-
Choline chloride[c]	0.05	0.05	0.05	0.05
Vitamin premix[d]	0.10	0.10	0.10	0.10
Mineral premix[d]	0.10	0.10	0.10	0.10
Chromic oxide	0.25	0.25	0.25	0.25

[a] As-fed basis.

[b] Contained P, 21.0%; Ca, 15.0%; F, 2.1 g/kg, and Fe, 9.0 g/kg; Supplied by Champion Feed Services Ltd, Westlock, AB, Canada.

[c] Contained 60% choline chloride; Supplied by Champion Feed Services Ltd, Westlock, AB, Canada.

[d] Supplied by Champion Feed Services Ltd, Westlock, AB, Canada. The mineral premix provided (per kilogram of diet): Fe, 135 mg; Zn, 135 mg; Mn, 40 mg; Cu, 20 mg; I, 0.5 mg; Co, 0.5 mg, and Se, 0.3 mg. The vitamin premix provided (per kilogram of diet): vitamin A, 12,000 IU; vitamin D_3, 1,500 IU; vitamin E, 50 IU; vitamin K_3, 1.5 mg; riboflavin, 5.5 mg; niacin, 25 mg; pantothenic acid, 15 mg, and vitamin B_{12}, 0.02 mg.

Table V-2. Chemical compositions of the experimental diets[a]

Phytate level	High-phytate		Low-phytate	
Phytase (FTU/kg)	0	1000	0	1000
Dry matter (%)	89.5	89.6	88.2	88.3
Gross energy (kcal/kg)	4,232	4,240	3,924	3,940
Metabolizable energy (kcal/kg)[b]	3,286	3,286	3,288	3,288
Crude protein (%)	18.73	18.77	18.10	17.82
Neutral detergent fiber (%)	26.4	26.2	19.6	19.4
Phosphorus (total, %)	0.77	0.77	0.51	0.52
Phosphorus (available, %)[b]	0.23	0.23	0.23	0.23
Calcium (%)	1.30	1.31	0.86	0.83
Ash (%)	7.23	7.14	5.23	5.20
Indispensable amino acids (%)				
Arginine	1.10	1.07	1.03	0.99
Histidine	0.43	0.43	0.40	0.42
Isoleucine	0.82	0.84	0.82	0.80
Leucine	1.53	1.52	1.49	1.45
Lysine	0.98	0.96	0.91	0.90
Methionine	0.19	0.21	0.21	0.19
Phenylalanine	0.87	0.85	0.85	0.83
Threonine	0.69	0.67	0.63	0.63
Valine	0.98	0.94	0.90	0.88
Dispensable amino acids (%)				
Alanine	0.96	0.96	0.86	0.86
Aspartic acid	1.70	1.61	1.58	1.63
Glutamic acid	3.80	3.58	3.48	3.60
Glycine	0.90	0.86	0.79	0.78
Serine	0.78	0.75	0.72	0.74
Tyrosine	0.45	0.48	0.46	0.44
Phytate-phosphorus (%)	0.478	0.483	0.221	0.224

Phytase activity (FTU/kg)	136	1740	115	1910

[a] As-fed basis.

[b] Calculated, based on NRC (1998).

Table V-3. Effect of phytase supplementation on the apparent ileal digestibilities (%) of energy, crude protein and amino acids (AA) in the high- and the low-phytate diets

Phytate level	High-phytate		Low-phytate			*P*-value[b]	
Phytase (FTU/kg)	0	1000	0	1000	SEM[a]	Dt-Ef[c]	Ps-Ef[d]
Energy	66.5[f]	65.1[f]	71.2[e]	71.0[e]	0.51	<0.0001	0.1530
Crude protein	71.9[f]	72.5[f]	74.8[e]	75.9[e]	0.43	<0.0001	0.0628
Indispensable AA							
Arginine	85.0	84.9	86.1	86.6	0.45	0.0077	0.6999
Histidine	81.7[f]	81.9[f]	84.8[e]	84.8[e]	0.77	0.0010	0.8580
Isoleucine	74.4[f]	75.4[f]	78.5[e]	79.1[e]	1.02	0.0012	0.4646
Leucine	77.4[f]	78.2[ef]	80.6[ef]	81.2[e]	0.92	0.0035	0.4772
Lysine	76.2	76.5	78.3	79.6	0.93	0.0122	0.4476
Methionine	81.4[f]	85.6[ef]	85.2[ef]	88.2[e]	1.46	0.0406	0.0229
Phenylalanine	77.2[f]	78.0[f]	81.1[e]	82.0[e]	0.89	0.0003	0.3691
Threonine	65.0	66.4	69.1	69.5	1.31	0.0142	0.5171
Valine	71.5[f]	72.6[ef]	75.6[e]	76.2[e]	1.06	0.0022	0.4530
Dispensable AA							
Alanine	71.7	72.9	74.7	74.6	0.87	0.0169	0.5317
Aspartic acid	70.4[f]	72.0[f]	75.2[e]	75.9[e]	0.96	0.0003	0.2698
Glutamic acid	82.0[f]	83.3[ef]	84.0[ef]	84.7[e]	0.67	0.0201	0.1458
Glycine	64.8	64.9	65.1	66.6	1.13	0.3976	0.4677
Serine	73.8[g]	74.6[fg]	76.8[ef]	77.6[e]	0.81	0.0015	0.3415
Tyrosine	76.0[f]	77.9[ef]	79.2[ef]	80.4[e]	0.93	0.0062	0.1092

[a] Standard error of the mean (n = 8).

[b] *P*-values obtained from the orthogonal contrasts.

[c] Effect of diet.

[d] Effect of phytase supplementation.

[e, f, g] Means within rows with different superscripts differ ($P < 0.05$).

Table V-4. Effect of phytase supplementation on the apparent fecal digestibilities (%) of energy and crude protein in the high- and the low-phytate diets

Phytate level	High-phytate		Low-phytate			*P*-value[b]	
Phytase (FTU/kg)	0	1000	0	1000	SEM[a]	Dt-Ef[c]	Ps-Ef[d]
Energy	83.0[f]	82.5[f]	88.2[e]	88.0[e]	0.37	<0.0001	0.3398
Crude protein	85.0[f]	85.6[f]	87.7[e]	87.9[e]	0.44	<0.0001	0.3885

[a] Standard error of the mean (n = 8).

[b] *P*-values obtained from the orthogonal contrasts.

[c] Effect of diet.

[d] Effect of phytase supplementation.

[e, f] Means within rows with different superscripts differ ($P < 0.05$).

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CHAPTER VI. GENERAL DISCUSSION, SUMMARY, AND PROSPECTS

A. Introduction

As was discussed by Liao et al. (2002), the very limited ability of pigs to utilize phytate phosphorus (**P**) in feedstuffs of plant origin poses three problems to swine producers, feed manufacturers, environmentalists, and the general public. The first problem involves the supplementation of inorganic P, which is expensive, to swine diets to meet the requirement of the pig for bioavailable P. Usually, inorganic P is over supplied to ensure that the requirement for bioavailable P is met. The second problem is the environmental P pollution resulting from excretion of a large proportion of P into manure. Modern intensive swine production operations are making this problem more serious, and growing concerns over this issue are globally expressed. The third problem relates to the ability of phytate, including phytic acid, to bind other nutrients, such as minerals, proteins, amino acids (**AA**) and starch.

The pigs' requirement for bioavailable P can be met by supplementation of diets with inorganic P. However, over supplementation to provide a safety margin can worsen the second problem. It is understood that the reason why pigs can not utilize phytate-P in feedstuffs of plant origin is that pigs do not produce phytase enzyme to hydrolyze phytate. Therefore, suitable strategies to solve the aforementioned problems are either to decrease the phytate content in feedstuffs or to increase phytase activities in the digestive tract. To address the potential P pollution, three types of strategy can be devised. The first type of strategy is to genetically modify some cultivars of crops to yield lower levels of phytate content in seeds (Allee, 2002). The second type of strategy is to supplement swine diets with exogenous phytase, which usually originates from microbes (Liao et al.,

2002). The third type of strategy is to develop transgenic pigs that can express endogenous phytase at an effective level of activity (Forsberg et al., 2003).

B. Strategies to Reduce Phosphorus in Swine Manure

Plant Breeding Strategies

In order to minimize environmental P pollution from swine and poultry production, plant breeders are developing new cultivars of crops, which contain less phytate-P than normal cultivars, by using both classical breeding and genetic engineering techniques (Satter and Wu, 2000; Raboy, 2002). Listed in Table VI-1 are some recently developed low-phytate crops including corn, barley and soybeans, which contain 2 to 5 times as much bioavailable P as normal crops which are the near isogenic lines. It is important to stress that the total P contents in the low-phytate crops are similar to or more than in the near isogenic normal crops (Etherton et al., 2003) There is no difference in performance between pigs fed the low-phytate and normal crops, and the P excretion in manure is reduced by 25 to 50%. Other nutrients in these low-phytate crops are equally available, or slightly more than in the normal crops (Allee, 2002).

In our laboratory, several experiments are being conducted to determine to what extent the excretion of P in manure can be reduced when pigs are fed low-phytate hulled or hulless barley, compared to normal hulled or hulless barley. These low-phytate barleys were developed by Dr. James H. Helm at the Field Crop Development Center, AAFRD, in Lacombe, Alberta. Preliminary results show that pigs fed low-phytate hulled barley excrete at least 50% less P than pigs fed normal hulled barley.

During the development of low-phytate crops, one should not forget that phytate serves several physiological functions in plants (Reddy et al., 1989; Ravindran et al., 1995), and that dietary phytate also has a beneficial role in human nutrition, for example as an antioxidant or anticancer agent (Graf, 1986; Raboy, 2002). There is a promising future for low-phytate crops if the following agronomic problems are solved. These problems include decreased germination rate, kernel size, and yield (e.g., Satter and Wu, 2000). Other field performance characteristics such as stress and disease tolerance have

not been fully investigated yet. Another unavoidable issue, in terms of food safety, is the acceptance of GMO food and feed by consumers, and this issue deserves public debate (Rossnagel, 2001).

Animal Breeding Strategies

Augmentation of the natural repertoire of digestive enzymes with phytase could, in principle, totally change the inability of pigs to hydrolyze phytate. In order to minimize environmental P pollution from the swine industry, a research group at the University of Guelph has generated 33 transgenic lines of Yorkshire pigs, trademarked Enviropig™, by microinjection of a constitutive *psp-appA* transgene into the pronuclear embryos of pigs. Constructed in the *psp-appA* transgene, the *appA* gene was originally cloned from *Escherichia coli* bacteria (Golovan et al., 2001). The Enviropig™ synthesizes phytase in its salivary glands. Salivary glands are suitable organs for phytase expression, since phytase in saliva can mix with diet as it is chewed and swallowed, and remains active in the stomach (Forsberg et al., 2003).

Studies with the offspring from two of the 33 transgenic lines, namely WA and JA, showed that pigs excrete up to 75% less P in feces than non-transgenic pigs (Table VI-2). In addition, there is almost no need for dietary supplementation of inorganic P (Golovan et al., 2001; Forsberg et al., 2003). Due to the advances in porcine transgenesis and cloning technology (Houdebine, 2003), a potential exists to introduce the *appA* gene into commercial herds of pigs.

The Enviropig™ could finally reach the consumer meat counter, assuming some "hurdles" can be overcome (Golovan et al. 2001). These "hurdles" include the issues of food safety, environmental regulation, public ethics, and animal welfare (Forsberg et al., 2003).

Animal Nutritional Strategies

Supplementation of swine diets with inorganic P is a basic measure to ensure that the pig obtains sufficient bioavailable P for bone development and optimal growth. To compensate for the variation in P content and bioavailability in feedstuffs, including inorganic P supplements, a safety margin for P is usually included in the diet. Surveys of

some commercial diets for sows and finishing pigs showed that the P contents were 40 and 55% higher, respectively, than the recommendations suggested by NRC (1988) (cited in NRC, 1998). This excessive amount of P will be excreted in manure. Therefore, from a nutritional point of view, precise matching the dietary supply of P with the requirement of the pig should be the first strategy to reduce P excretion, since this can be applied before feeding the pig. To apply this strategy, the safety margin for P needs to be reduced, and feed ingredients that are low in phytate should be selected. However, a precise determination of P bioavailabilities in a variety of feedstuffs and of P requirements of different categories of pigs is a real challenge for animal nutritionists.

Another important nutritional strategy is to supply the diets with exogenous phytase, which will assist the pig to hydrolyze phytate. Although it has been known for decades that the utilization of phytate-P by pigs is very poor, research on the supplementation of phytase to swine diets was only given impetus in the 1990's. One reason is directly related to the environmental P pollution from animal agriculture, which has become a political issue. The other reason is due to recent advances in biotechnology, which allow for large-scale commercial production of microbial phytase (Liao et al., 2002). It is expected that dietary supplementation of microbial phytase to improve the utilization of phytate-P by pigs will be an effective, realistic, and possibly long-term strategy to minimize enviromental P pollution from swine production, since, as was discussed previously, there are many biological problems, and societal, health and environmental concerns that exist with the generation of GMO crops and animals.

C. Phytase Strategy vs. Nutrient Utilization

Supplementation of phytase to swine diets has been widely accepted in Europe, but not in some other parts of the world. Estimates from a global perspective indicate that only about 8% of swine and poultry feeds are supplemented with phytase (Sheppy, 2001). It speaks for itself that phytase should be globally applied to animal diets, especially to diets for nonruminants, to alleviate the burden of P pollution to the environment. However, the application of phytase by the swine industry, at least in some parts of the

world, depends not only on its environmental consequence, but also on its benefits to the farm gate economy.

Understanding the effect of phytase supplementation on the utilization of nutrients other than P, including other minerals and AA, in swine diets may encourage swine producers and feed manufacturers to apply phytase to the diets, and challenge the feed industry to reconsider their traditional assumptions, in diet formulation practices, about ingredient selection, nutrient requirements, and desired responses in animal performance. However, results reported in the literature from a few studies on the effect of phytase supplementation on the utilization of other nutrients, especially AA, are inconsistent (Liao et al., 2002; Adeola and Sands, 2003).

The objective of this research project was to evaluate the effect of phytase supplementation on the utilization of various nutrients and energy in different swine diets. The project was divided into two phases. In phase 1, four experiments were conducted with four different starter diets fed to weanling pigs. The diets were formulated with various feed ingredients that are commonly used in western Canada. In phase 2, one experiment was conducted with two model diets fed to growing pigs. The diets were formulated to contain a high and a low content of phytate. The high-phytate diet contained 20% rice bran which is a rich source of phytate-P. The results obtained from these experiments were reported in the previous chapters and are summarized as follows.

Effect on Phosphorus Balance

Previous investigations have shown that supplementation of phytase to swine diets improves the digestibility and retention of P (Cromwell et al., 1993; Lei et al., 1993a, c). However, most of studies were carried out with corn-soybean meal diets without or with little inorganic P supplementation. In this project, P balance studies were conducted with six different diets. Results reported in Chapter II showed that phytase supplementation to four diets for weanling pigs improved ($P < 0.001$) the apparent fecal digestibility (**AFD**) of P by 6.5 to 12.7 percentage units (**pu**); the fecal output of P was reduced ($P < 0.05$) by 9.0 to 30.2% (Table II-4). The daily urinary output of P tended to increase ($P < 0.10$) after phytase supplementation to three of the four diets. Compared to the fecal output,

the urinary output of P is of a very small magnitude. The retention of P was improved ($P < 0.05$) by 12.7 to 25.6% upon phytase supplementation.

Results reported in Chapter IV showed that phytase supplementation improved ($P < 0.001$) the AFD of P by 11.4 and 19.6 pu in the high- and the low-phytate diets, respectively; the fecal outputs of P were reduced ($P < 0.001$) by 14.9 and 30.5%, respectively (Table IV-3). The magnitudes of increase in the AFD of P or of decrease in the fecal output of P upon phytase supplementation were not larger ($P > 0.10$) in the high- compared to the low-phytate diet. The daily urinary output of P increased ($P = 0.03$) after phytase supplementation to both diets. The retentions of P were improved ($P < 0.05$) by 44.4 and 50.2% for the high- and the low-phytate diets, respectively. The magnitudes of increase in the daily P retention for the high-phytate diet upon phytase supplementation were not larger ($P < 0.10$) than for the low-phytate diet.

These results show that the supplementation of phytase to diets formulated with commonly used feed ingredients, even with inorganic P supplementation, will improve the AFD and decrease the fecal excretion of P in weanling and growing pigs. The improvements in P retention in these studies imply that the requirements for bioavailable P, as suggested by NRC (1998) for pigs, may be too low.

Effect on Calcium Balance

Associated with P, calcium (Ca) also plays a major role in the development and maintenance of the skeletal system and performs many other physiological functions. Although Ca has the lowest binding affinity to phytic acid *in vitro*, the greatest impact of phytate on mineral nutrition other than P is on Ca utilization since it is the dominant mineral in most diets (Wise, 1983; Ravindran et al., 1995). Many previous studies have shown that supplementation of phytase to swine diets can improve Ca digestibility and/or retention (Jongbloed et al., 1993; 2000). However, results obtained in this project showed that the effect of phytase supplementation on the digestibility and retention of Ca varies with the composition of the diets.

As reported in Chapter II, phytase supplementation increased ($P < 0.05$) the AFD of Ca in weanling pigs fed the wheat-soybean meal and the barley-peas-canola meal diets, but not ($P > 0.10$) in weanling pigs fed the corn-soybean meal and the wheat-soybean

meal-canola meal diets (Table II-5). The magnitudes of improvement ($P < 0.05$) in weanling pigs fed the wheat-soybean meal and wheat-soybean meal-canola meal diets ranged from 8.6 to 8.9 pu. The fecal output of Ca in weanling pigs fed the wheat-soybean meal diet was reduced significantly ($P < 0.05$). There were no differences ($P > 0.10$) in the daily urinary outputs of Ca before and after phytase supplementation. The retention of Ca was only increased ($P < 0.05$) when phytase was supplemented to the wheat-soybean meal diet, but not to the other three diets.

As reported in Chapter IV, phytase supplementation increased ($P < 0.05$) the AFD and also the apparent ileal digestibility (**AID**) of Ca for the low-phytate diet, but not ($P > 0.10$) for the high-phytate diet (Table IV-3). The magnitudes of increase in the AFD and also the AID of Ca for the low-phytate diet were higher ($P < 0.05$) than for the high-phytate diet. The daily urinary output of Ca was decreased ($P < 0.001$) upon phytase supplementation to both diets. There were increases ($P < 0.05$) in the daily Ca retention upon phytase supplementation to the diets. The magnitudes of increases upon phytase supplementation were not different ($P < 0.10$) between the high- and the low-phytate diet.

The data presented in Chapters II and IV showed that a positive response to phytase supplementation on Ca utilization occurred when the diets were relatively low in Ca. The dietary Ca levels were 0.84 and 0.70% in the wheat-soybean meal and the barley-peas-canola meal diets, respectively, and 0.94 and 1.05% in the corn-soybean meal and the wheat-soybean meal-canola meal diets, respectively (Table II-3). The Ca level was 0.85% in the low-phytate diet, and 1.31% in the high-phytate diet (Table IV-2). Our observation is in agreement with results reported by Jongbloed et al. (1993), who showed that the largest increase in the AFD of Ca was obtained from the diet with the lowest content of Ca and the highest rate of phytase supplementation.

Effect on Digestibilities of Other Minerals

Phytate in feedstuffs may bind other minerals (polyvalent cations) in the diets, forming insoluble phytate-mineral complexes (Adeola et al, 1995; Szkudelski, 1995; Jongbloed et al., 2000). Supplementation of phytase to swine diets makes it plausible that these minerals are liberated and their bioavailabilities improved as a result of hydrolysis of the orthophosphate groups from the phytate molecules. Results reported in

the literature on the effect of phytase supplementation on the AFD of these minerals are contradictory (Näsi, 1990; Pallauf et al., 1992; Lei et al., 1993b; Näsi and Helander, 1994; Adeola et al., 1995; Ashida et al. 1999).

The AFD of copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), and zinc (Zn) were determined in this project. As reported in Chapter II, numerical increases ($P > 0.10$) in the AFD of Cu, Mg, Mn, and Zn were detected upon phytase supplementation, but not in the AFD of Fe (Table II-6). In Chapter IV, it was reported that phytase supplementation did not affect ($P > 0.10$) the AFD of Cu, Fe, Mg, Mn, and Mo, and that the AFD of Zn was decreased ($P < 0.05$) upon phytase supplementation to the high-phytate diet (Table IV-4). It was concluded that the changes in the AFD of these minerals upon phytase supplementation can not simply be predicted from the changes in the AFD of P or Ca. Supplementation of minerals (via the premix) which exceeded the requirements of the pig, and the unknown interactions among dietary minerals may explain the results obtained so far. In short, the effect of phytase supplementation on the utilization of minerals, other than P and Ca, is negligible.

Effect on Digestibilities of Amino Acids

The cost-effectiveness of phytase supplementation to swine diets would be improved if it were established that phytase has a positive effect on the utilization of AA. Results reported in the literature on the utilization of AA are inconsistent (Liao et al., 2002; Adeola and Sands, 2003). The effect of phytase supplementation to different diets for weanling and growing pigs on the AID of CP and AA was another focus of this research project.

Results reported in Chapter III showed that no significant improvements ($P > 0.05$) in the AID of CP and AA were detected upon phytase supplementation to the four diets fed to the weanling pigs; however, small numerical increases in the AID of CP and AA were found in all experiments (Tables III-4, III-5, III-6 and III-7). Occasionally, some increases in some AA approached significance ($P < 0.10$). Numerically, the AID of CP was increased by 0.2 to 2.8 pu upon phytase supplementation, and the average of the AID of the indispensable AA was increased ($P > 0.05$) by 0.5 to 2.9 pu (Table III-10). These

results are in agreement with most studies reported in the literature (Kies et al., 1997; Liao et al., 2002).

Results reported in Chapter V showed that phytase supplementation to the high- and low-phytate diets did not improve ($P > 0.10$) the AID of AA in growing pigs, although there was a trend ($P < 0.10$) towards an improvement in the AID of CP (Table V-3). Consistent with results reported in Chapter III, there were small numerical increases in the AID of CP and AA. Numerically, the AID of CP was increased ($P > 0.05$) by 0.6 pu for the high-phytate diet, and by 1.1 pu for the low-phytate diet. The average of the AID of the indispensable AA was increased ($P > 0.05$) by 0.6 pu for both the high- and the low-phytate diets.

Based on the results obtained in this project, it can be concluded that any "AA response factor" or "AA equivalency value" to dietary phytase supplementation, which was ascribed by some investigators, is still overly simplistic at present, as was also pointed out by Adeola and Sands (2003).

Effect on Other Parameters

The effect of phytase supplementation on the AFD of ash, DM, and energy was also determined in this project. In phase 1 of this project, although the increase in the AFD of ash upon phytase supplementation to the corn-soybean meal diet did not reach significance ($P > 0.10$), the AFD of ash were significantly improved ($P < 0.05$) in the other three diets (Table II-7). In phase 2 of this project, the AFD of ash was increased ($P < 0.05$) for the low-phytate diet, and there was a trend ($P < 0.10$) for an increase for the high-phytate diet (Table IV-5). In general, the AFD of ash is improved upon phytase supplementation to swine diets.

As reported in Chapter II, the increases in the AFD of DM approached significance ($P < 0.10$) with the wheat-soybean meal and the barley-peas-canola meal diets, but not ($P > 0.10$) with the corn-soybean meal and the wheat-soybean meal-canola meal diets fed to weanling pigs. It was reported in Chapter IV that phytase supplementation to both high- and low-phytate diets for growing pigs did not ($P > 0.10$) result in an improvement in the AFD of DM (Table IV-5). In short, the response to phytase supplementation in terms of AFD of DM is variable.

There were no differences ($P > 0.10$) in the AFD of energy between the phytase-supplemented and non-supplemented diets in all experiments, although some numerical increases ($P = 0.16$ to 0.54) were found upon phytase supplementation to the diets fed to the weanling pigs.

In conclusion, the increases in the AFD of P and Ca upon phytase supplementation can improve the AFD of ash. The numerical increases in the AID or AFD of CP and AA are too small to result in an increase in the AFD of energy. Phytase supplementation may increase the AFD of DM.

D. Prospects for Future Research

An extensive review of the literature (Chapter I) and the results from five experiments (Chapters II to V) suggest that there are many factors and interactions among dietary components that influence the effect of phytase supplementation on the utilization of different nutrients. It is understandable to find different responses in terms of utilization of different nutrients when phytase is supplemented to different diets. A key issue now is how to predict these responses for different parameters with different diets based on the information from specific feed ingredients.

As was discussed in Chapter I, dietary factors that govern or interact with the response to phytase supplementation include the dietary contents of phytate, different minerals, vitamins (e.g., vitamin D), intrinsic phytase activity, and some anti-nutritive factors. Clearly, the interactions among these dietary factors are a multifaceted subject which merit further studies. For further research, opportunities exist to identify these factors and interactions, and quantify their effects on the magnitudes of responses to phytase. Following, linear or non-linear computer models should be developed to accurately predict animal responses to phytase for different parameters with different diets. The data generated from the computer models would not only encourage the swine industry to increase the application of phytase, but also assist feed manufacturers to more accurately formulate diets that will precisely match requirements with dietary supply.

The benefit of dietary supplementation of phytase in reducing P output from the swine industry has been obvious. However, the effect of phytase supplementation on the

utilization of other nutrients is not guaranteed yet, in light of current knowledge. It still seems impossible to exceed P bioavailabilities of 60 to 70% in feed ingredients of plant origin, even if phytase is supplemented at a very high rate. Current use of commercially available phytase as a routine feed additive is also limited by inactivation of phytase at the high temperature (65 to 80°C) required for steam-pelleting, and by loss of activity during long-term storage/transport at ambient temperatures (Lei and Stahl, 2001). Therefore, for the enzyme industry there are also opportunities that exist to improve the efficacy or quality of their phytase products.

Ideal phytase should have a high catalytic activity, broad substrate specificity, high thermo-stability, good resistance to proteolysis, high residual activity within a pH range of 2.5 to 7.7, inexpensive to produce, and easy to use (Lei and Stahl, 2001; Zyla, 2001). However, any single phytase enzyme may never be "ideal" for all categories of pigs or in all cases of application. Thereby, a series of phytases for different pigs with different physiological status and feeding conditions should be specifically identified or designed (Rodriguez et al., 2000b; Lei and Stahl, 2001). The search for new desirable phytase enzymes should be continually pursued (Matsui et al., 2000; Stahl et al., 2000). Furthermore, as no single protein expression system is likely to be able to produce a series of phytase enzymes, developing more cost-effective expression systems should also be continued (Rodriguez et al., 2000a, b; Stahl et al., 2003).

Table VI-1. Phosphorus contents (%) of low-phytate and normal crops[a, b]

Crops	Low-phytate crops	Normal crops
Corn		
Total phosphorus	0.28	0.25
Phytate phosphorus	0.10	0.20
Non-phytate phosphorus	0.18	0.05
Barley		
Total phosphorus	0.35	0.35
Phytate phosphorus	0.14	0.24
Non-phytate phosphorus	0.21	0.11
Soybeans		
Total phosphorus	0.57 ~ 0.65	0.63
Phytate phosphorus	0.16 ~ 0.18	0.43
Non-phytate phosphorus	0.41 ~ 0.47	0.20

[a] As-fed basis.

[b] Allee (2002).

Table VI-2. True phosphorus digestibility (%) in a transgenic phytase pig line WA fed soybean meal as the sole source of phosphorus (Golovan et al., 2001)[a]

Pigs	Non-transgenic pigs	Transgenic pigs
Weanling pigs	48.5 ± 5.4^{b} (n = 16)	87.9 ± 3.4^{c} (n = 14)
Growing-finishing pigs	51.9 ± 10.3^{b} (n = 16)	98.8 ± 3.4^{c} (n = 14)

[a] The digestibility values were obtained with the regression analysis technique.

[b, c] Mean ± SEM; Values in the same row with different superscripts differ ($P < 0.01$).

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